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(54) **DELAMINATION RESISTANT PHARMACEUTICAL GLASS CONTAINERS CONTAINING ACTIVE PHARMACEUTICAL INGREDIENTS**

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(57) **ABSTRACT**

The present invention is based, at least in part, on the identification of a pharmaceutical container formed, at least in part, of a glass composition which exhibits a reduced propensity to delaminate, i.e., a reduced propensity to shed glass particulates. As a result, the presently claimed containers are particularly suited for storage of pharmaceutical compositions and, specifically, a pharmaceutical solution comprising a pharmaceutically active ingredient, for example, GAMMAGARD LIQUID (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant), Plasma/Albumin-Free Method, (rAHF)); BAX 111 (vonicog alfa, or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or an adeno-associated viral vector containing a liver-specific human factor IX expression cassette, e.g., sscAAV2/8-LP1-hFIXco.

12 Claims, 4 Drawing Sheets

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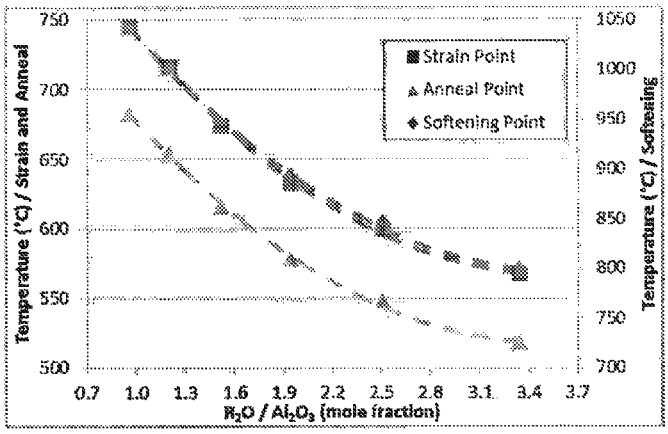
Strain Point
Anneal Point
Softening Point
Temperature (°C) / Strain and Anneal
Temperature (°C) / Softening
R_2O / Al_2O_3 (mole fraction)

FIG. 1

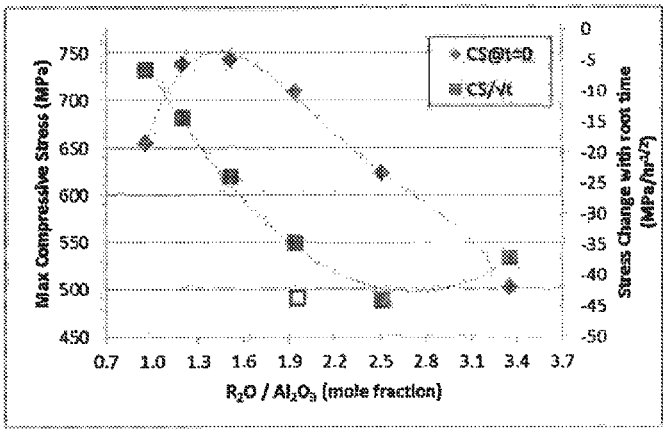
CS@t=0
Max Compressive Stress (MPa)
Stress Change with root time (MPa/hr$^{1/2}$)
R_2O / Al_2O_3 (mole fraction)

FIG. 2

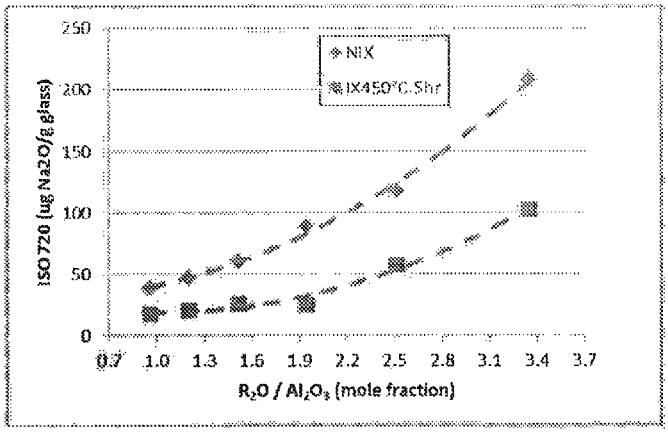
250
200
150
100
50
0
ISO 720 (ug Na2O/g glass)
NIK
IX450°C. 5hr
0.7 1.0 1.3 1.6 1.9 2.2 2.5 2.8 3.1 3.4 3.7
R2O / Al2O3 (mole fraction)

FIG. 3

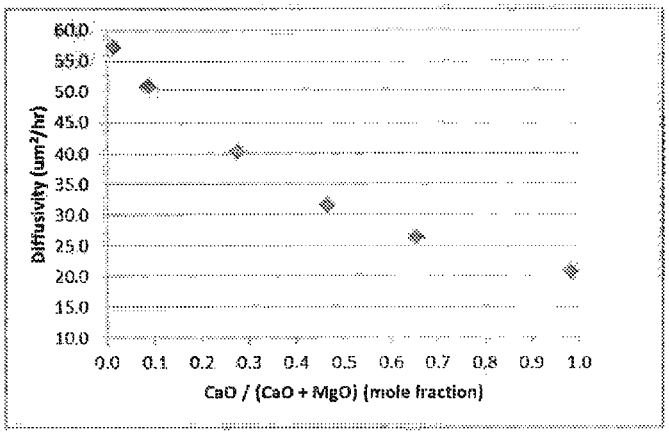
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
Diffusivity (um²/hr)
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
CaO / (CaO + MgO) (mole fraction)

FIG. 4

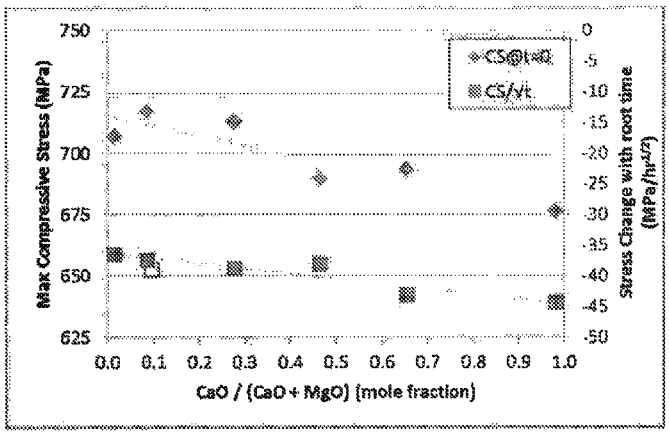
Max Compressive Stress (MPa)
750
725
700
675
650
625
Stress Change with root time (MPa/hr^1/2)
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
CS@t=0
CS/√t
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
CaO / (CaO + MgO) (mole fraction)

FIG. 5

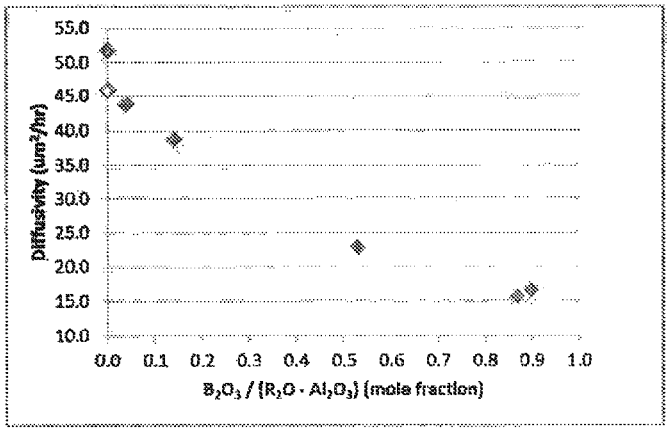
Diffusivity (um²/hr)
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
B2O3 / (R2O + Al2O3) (mole fraction)

FIG. 6

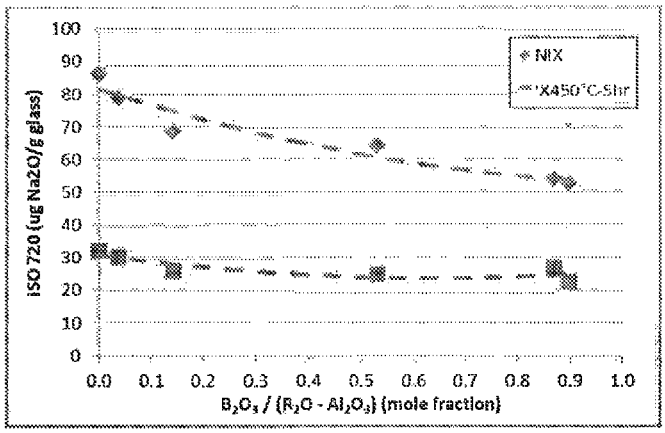
100
90
80
70
60
50
40
30
20
10
0
ISO 720 (ug Na2O/g glass)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
B2O3 / (R2O - Al2O3) (mole fraction)
NIX
X450°C-5hr

FIG. 7

DELAMINATION RESISTANT PHARMACEUTICAL GLASS CONTAINERS CONTAINING ACTIVE PHARMACEUTICAL INGREDIENTS

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Application No. 61/815,658, filed Apr. 24, 2013, entitled "Delamination Resistant Pharmaceutical Glass Containers Containing Active Pharmaceutical Ingredients", the entirety of which is hereby incorporated by reference herein.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Apr. 21, 2014, is named 122467-01802_SL.txt and is 31,160 bytes in size.

FIELD OF THE INVENTION

The present specification generally relates to pharmaceutical containers and, more specifically, to chemically and mechanically durable pharmaceutical containers that are delamination resistant and formed, at least in part, of a glass composition.

BACKGROUND

The design of a packaged pharmaceutical composition generally seeks to provide an active pharmaceutical ingredient (API) in a suitable package that is convenient to use, that maintains the stability of the API over prolonged storage, and that ultimately allows for the delivery of efficacious, stable, active, nontoxic and nondegraded API.

Most packaged formulations are complex physico-chemical systems, through which the API is subject to deterioration by a variety of chemical, physical, and microbial reactions. Interactions between drugs, adjuvants, containers, and/or closures may occur, which can lead to the inactivation, decomposition and/or degradation of the API.

Historically, glass has been used as the preferred material for packaging pharmaceuticals because of its hermeticity, optical clarity and excellent chemical durability relative to other materials. Specifically, the glass used in pharmaceutical packaging must have adequate chemical durability so as not to affect the stability of the pharmaceutical compositions contained therein. Glasses having suitable chemical durability include those glass compositions within the ASTM standard 'Type 1B' glass compositions which have a proven history of chemical durability.

However, use of glass for such applications is limited by the mechanical performance of the glass. Specifically, in the pharmaceutical industry, glass breakage is a safety concern for the end user as the broken package and/or the contents of the package may injure the end user. Further, non-catastrophic breakage (i.e., when the glass cracks but does not break) may cause the contents to lose their sterility which, in turn, may result in costly product recalls.

One approach to improving the mechanical durability of the glass package is to thermally temper the glass package. Thermal tempering strengthens glass by inducing a surface compressive stress during rapid cooling after forming. This technique works well for glass articles with flat geometries

(such as windows), glass articles with thicknesses >2 mm, and glass compositions with high thermal expansion. However, pharmaceutical glass packages typically have complex geometries (vial, tubular, ampoule, etc.), thin walls (~1-1.5 mm), and are produced from low expansion glasses (30–55×10^{-7} K^{-1}) making glass pharmaceutical packages unsuitable for strengthening by thermal tempering.

Chemical tempering also strengthens glass by the introduction of surface compressive stress. The stress is introduced by submerging the article in a molten salt bath. As ions from the glass are replaced by larger ions from the molten salt, a compressive stress is induced in the surface of the glass. The advantage of chemical tempering is that it can be used on complex geometries, thin samples, and is relatively insensitive to the thermal expansion characteristics of the glass substrate. However, glass compositions which exhibit a moderate susceptibility to chemical tempering generally exhibit poor chemical durability and vice-versa.

Finally, glass compositions commonly used in pharmaceutical packages, e.g., Type 1a and Type 1b glass, further suffer from a tendency for the interior surfaces of the pharmaceutical package to shed glass particulates or "delaminate" following exposure to pharmaceutical solutions. Such delamination often destabilizes the active pharmaceutical ingredient (API) present in the solution, thereby rendering the API therapeutically ineffective or unsuitable for therapeutic use.

Delamination has caused the recall of multiple drug products over the last few years (see, for example, Reynolds et al., (2011) BioProcess International 9(11) pp. 52-57). In response to the growing delamination problem, the U.S. Food and Drug Administration (FDA) has issued an advisory indicating that the presence of glass particulate in injectable drugs can pose a risk.

The advisory states that, "[t]here is potential for drugs administered intravenously that contain these fragments to cause embolic, thrombotic and other vascular events; and subcutaneously to the development of foreign body granuloma, local injections site reactions and increased immunogenicity."

Accordingly, a recognized need exists for alternative glass containers for packaging of pharmaceutical compositions which exhibit a reduced propensity to delaminate.

SUMMARY

In one aspect, the present invention includes a delamination resistant pharmaceutical container including a glass composition. The pharmaceutical container includes from about 70 mol. % to about 80 mol. % SiO_2; from about 3 mol. % to about 13 mol. % alkaline earth oxide; X mol. % Al_2O_3; and Y mol. % alkali oxide. The alkali oxide includes Na_2O in an amount greater than about 8 mol. %, a ratio of Y:X is greater than 1, and the glass composition is free of boron and compounds of boron. The delamination resistant pharmaceutical container further includes an active pharmaceutical ingredient.

In one or more embodiments, the SiO_2 is present in an amount less than or equal to 78 mol. %. In some embodiments, an amount of the alkaline earth oxide is greater than or equal to about 4 mol. % and less than or equal to about 8 mol. %. In one or more embodiments, the alkaline earth oxide includes MgO and CaO and a ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) is less than or equal to 0.5. In one or more embodiments, the alkaline earth oxide includes from about 0.1 mol. % to less than or equal to about 1.0 mol. % CaO. In one or more embodiments, the alkaline

earth oxide includes from about 3 mol. % to about 7 mol. % MgO. In one or more embodiments, X is greater than or equal to about 2 mol. % and less than or equal to about 10 mol. %. In embodiments, the alkali oxide includes greater than or equal to about 9 mol. % Na_2O and less than or equal to about 15 mol. % Na_2O. In some embodiments, the ratio of Y:X is less than or equal to 2. In one or more embodiments, the ratio of Y:X is greater than or equal to 1.3 and less than or equal to 2.0. In one or more embodiments, the alkali oxide further includes K_2O in an amount less than or equal to about 3 mol. %. In one or more embodiments, the glass composition is free of phosphorous and compounds of phosphorous. In one or more embodiments, the alkali oxide includes K_2O in an amount greater than or equal to about 0.01 mol. % and less than or equal to about 1.0 mol. %.

In another aspect, the invention includes a delamination resistant pharmaceutical container including a pharmaceutical composition. The pharmaceutical container includes an active pharmaceutical ingredient, such that the pharmaceutical container includes a glass composition including SiO_2 in a concentration greater than about 70 mol. %; alkaline earth oxide including MgO and CaO, wherein CaO is present in an amount greater than or equal to about 0.1 mol. % and less than or equal to about 1.0 mol. %, and a ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) is less than or equal to 0.5; and Y mol. % alkali oxide, wherein the alkali oxide includes Na_2O in an amount greater than about 8 mol. %, such that the glass composition is free of boron and compounds of boron

In another aspect, the invention includes a delamination resistant pharmaceutical container including a pharmaceutical composition including an active pharmaceutical ingredient. The pharmaceutical container includes a glass composition including from about 72 mol. % to about 78 mol. % SiO_2; from about 4 mol. % to about 8 mol. % alkaline earth oxide, wherein the alkaline earth oxide includes MgO and CaO and a ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) is less than or equal to 0.5; X mol. % Al_2O_3, such that X is greater than or equal to about 4 mol. % and less than or equal to about 8 mol. %; and Y mol. % alkali oxide, such that the alkali oxide includes Na_2O in an amount greater than or equal to about 9 mol. % and less than or equal to about 15 mol. %, a ratio of Y:X is greater than 1, and the glass composition is free of boron and compounds of boron.

In another aspect, the invention includes a delamination resistant pharmaceutical container including a pharmaceutical composition including an active pharmaceutical ingredient. The pharmaceutical container includes a glass composition. The glass composition includes from about 70 mol. % to about 80 mol. % SiO_2; from about 3 mol. % to about 13 mol. % alkaline earth oxide, such that the alkaline earth oxide includes CaO in an amount greater than or equal to about 0.1 mol. % and less than or equal to about 1.0 mol. %, MgO, and a ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) is less than or equal to 0.5; X mol. % Al_2O_3, wherein X is greater than or equal to about 2 mol. % and less than or equal to about 10 mol. %; and Y mol. % alkali oxide, wherein the alkali oxide includes from about 0.01 mol. % to about 1.0 mol. % K_2O and a ratio of Y:X is greater than 1, and the glass composition is free of boron and compounds of boron.

In one or more embodiments of any of the above aspects, the pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T, 1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette.

In one aspect, the present invention includes a pharmaceutical composition. The pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette and a pharmaceutically acceptable excipient, such that the pharmaceutical composition is contained within a glass pharmaceutical container including an internal homogeneous layer.

In one or more embodiments, the pharmaceutical container has a compressive stress greater than or equal to 150 MPa. In one or more embodiments, the pharmaceutical container has a compressive stress greater than or equal to 250 MPa. In one or more embodiments, the pharmaceutical container includes a depth of layer greater than 30 μm. In one or more embodiments, the depth of layer is greater than 35 μm. In one or more embodiments, the pharmaceutical composition demonstrates increased stability, product integrity, or efficacy.

In one aspect, the present invention includes a pharmaceutical composition. The pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette and a pharmaceutically acceptable excipient, such that the pharmaceutical composition is contained within a glass pharmaceutical container including an internal homogeneous layer having a compressive stress greater than or equal to 150.

In one or more embodiments, the pharmaceutical container includes a depth of layer greater than 10 μm. In one or more embodiments, the pharmaceutical container includes a depth of layer greater than 25 μm. In one or more embodiments, the pharmaceutical container includes a depth of layer greater than 30 μm. In one or more embodiments, the pharmaceutical container has a compressive stress greater than or equal to 300 MPa. In one or more embodiments, the pharmaceutical container includes increased stability, product integrity, or efficacy.

In another aspect, the present technology includes a pharmaceutical composition. The pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette and a pharmaceutically acceptable excipient, such that the pharmaceutical composition is contained within a glass pharmaceutical container having a compressive stress greater than or equal to 150 MPa and a depth of layer greater

than 10 μm, and such that the pharmaceutical composition demonstrates increased stability, product integrity, or efficacy.

In another aspect, the present technology includes a pharmaceutical composition. The pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette and a pharmaceutically acceptable excipient, such that the pharmaceutical composition is contained within a glass pharmaceutical container including a substantially homogeneous inner layer, and such that the pharmaceutical composition demonstrates increased stability, product integrity, or efficacy.

In another aspect, the present technology includes a pharmaceutical composition. The pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette and a pharmaceutically acceptable excipient, such that the pharmaceutical composition is contained within a glass pharmaceutical container having a delamination factor of less than 3, wherein the pharmaceutical composition demonstrates increased stability, product integrity, or efficacy.

In another aspect, the present technology includes a pharmaceutical composition. The pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette and a pharmaceutically acceptable excipient, such that the pharmaceutical composition is contained within a glass pharmaceutical container which is substantially free of boron, and such that the pharmaceutical composition demonstrates increased stability, product integrity, or efficacy.

In one or more embodiments, the glass pharmaceutical container has a compressive stress greater than 150 MPa and a depth of layer greater than 25 μm. In one or more embodiments, the glass pharmaceutical container has a compressive stress greater than 300 MPa and a depth of layer greater than 35 μm. In one or more embodiments, the glass pharmaceutical container includes a substantially homogeneous inner layer. In one or more embodiments, the glass pharmaceutical container has a compressive stress greater than or equal to 150 MPa and a depth of layer greater than 25 μm.

In another aspect, the present technology includes a pharmaceutical composition. The pharmaceutical composition includes GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette and a pharmaceutically acceptable excipient, such that the pharmaceutical composition is contained within a glass pharmaceutical container including a delamination factor of less than 3, and such that the pharmaceutical composition includes increased stability, product integrity, or efficacy.

In one or more embodiments of any of the above aspects, the container has a compressive stress greater than 300 MPa. In one or more embodiments, the container has a depth of layer greater than 25 μm. In one or more embodiments, the container has a depth of layer greater than 30 μm. In one or more embodiments, the container has a depth of layer of at least 35 μm. In one or more embodiments, the container has a compressive stress greater than or equal to 300 MPa. In one or more embodiments, the container has a compressive stress greater than or equal to 350 MPa.

Additional features and advantages will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the embodiments described herein, including the detailed description which follows, the claims, as well as the appended drawings.

It is to be understood that both the foregoing general description and the following detailed description describe various embodiments and are intended to provide an overview or framework for understanding the nature and character of the claimed subject matter. The accompanying drawings are included to provide a further understanding of the various embodiments, and are incorporated into and constitute a part of this specification. The drawings illustrate the various embodiments described herein, and together with the description serve to explain the principles and operations of the claimed subject matter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** graphically depicts the relationship between the ratio of alkali oxides to alumina α-axis) and the strain point, annealing point, and softening point (y-axes) of inventive and comparative glass compositions;

FIG. **2** graphically depicts the relationship between the ratio of alkali oxides to alumina α-axis) and the maximum compressive stress and stress change (y-axes) of inventive and comparative glass compositions;

FIG. **3** graphically depicts the relationship between the ratio of alkali oxides to alumina α-axis) and hydrolytic resistance as determined from the ISO 720 standard (y-axis) of inventive and comparative glass compositions;

FIG. **4** graphically depicts diffusivity D (y-axis) as a function of the ratio (CaO/(CaO+MgO)) (x-axis) for inventive and comparative glass compositions;

FIG. **5** graphically depicts the maximum compressive stress (y-axis) as a function of the ratio (CaO/(CaO+MgO)) (x-axis) for inventive and comparative glass compositions;

FIG. **6** graphically depicts diffusivity D (y-axis) as a function of the ratio (B_2O_3/(R_2O—Al_2O_3)) (x-axis) for inventive and comparative glass compositions; and

FIG. **7** graphically depicts the hydrolytic resistance as determined from the ISO 720 standard (y-axis) as a function of the ratio (B_2O_3/(R_2O—Al_2O_3)) (α-axis) for inventive and comparative glass compositions.

DETAILED DESCRIPTION

The present invention is based, at least in part, on the identification of a pharmaceutical container formed, at least

in part, of a glass composition which exhibits a reduced propensity to delaminate, i.e., a reduced propensity to shed glass particulates. As a result, the presently claimed containers are particularly suited for storage, maintenance and/or delivery of therapeutically efficacious pharmaceutical compositions and, in particular pharmaceutical solutions comprising active pharmaceutical ingredients, for example, GAMMAGARD LIQUID or GAMMAGARD S/D (an immune globulin infusion (human)); ADVATE (Antihemophilic Factor (Recombinant)); BAX 111 (vonicog alfa, recombinant von Willebrand factor (rVWF) or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); and an adeno-associated viral vector containing a liver-specific human factor IX expression cassette, e.g., sscAAV2/8-LP1-hFIXco.

Conventional glass containers or glass packages for containing pharmaceutical compositions are generally formed from glass compositions which are known to exhibit chemical durability and low thermal expansion, such as alkali borosilicate glasses. While alkali borosilicate glasses exhibit good chemical durability, container manufacturers have sporadically observed silica-rich glass flakes dispersed in the solution contained in the glass containers as a result of delamination, particularly when the solution has been stored in direct contact with the glass surface for long time periods (months to years).

Delamination refers to a phenomenon in which glass particles are released from the surface of the glass following a series of leaching, corrosion, and/or weathering reactions. In general, the glass particles are silica-rich flakes of glass which originate from the interior surface of the package as a result of the leaching of modifier ions into a solution contained within the package. These flakes may generally be from about 1 nm to 2 μm thick with a width greater than about 50 μm.

It has heretofore been hypothesized that delamination is due to the phase separation which occurs in alkali borosilicate glasses when the glass is exposed to the elevated temperatures used for reforming the glass into a container shape.

However, it is now believed that the delamination of the silica-rich glass flakes from the interior surfaces of the glass containers is due to the compositional characteristics of the glass container in its as-formed condition. Specifically, the high silica content of alkali borosilicate glasses increases the melting temperature of the glass. However, the alkali and borate components in the glass composition melt and/or vaporize at much lower temperatures. In particular, the borate species in the glass are highly volatile and evaporate from the surface of the glass at the high temperatures necessary to melt and form the glass.

Specifically, glass stock is reformed into glass containers at high temperatures and in direct flames. The high temperatures cause the volatile borate species to evaporate from portions of the surface of the glass. When this evaporation occurs within the interior volume of the glass container, the volatilized borate species are re-deposited in other areas of the glass causing compositional heterogeneities in the glass container, particularly with respect to the bulk of the glass container. For example, as one end of a glass tube is closed to form the bottom or floor of the container, borate species may evaporate from the bottom portion of the tube and be re-deposited elsewhere in the tube. As a result, the areas of the container exposed to higher temperatures have silica-rich surfaces. Other areas of the container which are amenable to boron deposition may have a silica-rich surface with a boron-rich layer below the surface. Areas amenable to boron deposition are at a temperature greater than the anneal point of the glass composition but less than the hottest temperature the glass is subjected to during reformation when the boron is incorporated into the surface of the glass. Solutions contained in the container may leach the boron from the boron-rich layer. As the boron-rich layer is leached from the glass, the silica-rich surface begins to spall, shedding silica-rich flakes into the solution.

Definitions

The term "softening point," as used herein, refers to the temperature at which the viscosity of the glass composition is $1\times10^{7.6}$ poise.

The term "annealing point," as used herein, refers to the temperature at which the viscosity of the glass composition is 1×10^{13} poise.

The terms "strain point" and "T_{strain}" as used herein, refers to the temperature at which the viscosity of the glass composition is 3×10^{14} poise.

The term "CTE," as used herein, refers to the coefficient of thermal expansion of the glass composition over a temperature range from about room temperature (RT) to about 300° C.

In the embodiments of the glass compositions described herein, the concentrations of constituent components (e.g., SiO_2, Al_2O_3, and the like) are specified in mole percent (mol. %) on an oxide basis, unless otherwise specified.

The terms "free" and "substantially free," when used to describe the concentration and/or absence of a particular constituent component in a glass composition, means that the constituent component is not intentionally added to the glass composition. However, the glass composition may contain traces of the constituent component as a contaminant or tramp in amounts of less than 0.01 mol. %.

The term "chemical durability," as used herein, refers to the ability of the glass composition to resist degradation upon exposure to specified chemical conditions. Specifically, the chemical durability of the glass compositions described herein was assessed according to three established material testing standards: DIN 12116 dated March 2001 and entitled "Testing of glass—Resistance to attack by a boiling aqueous solution of hydrochloric acid—Method of test and classification"; ISO 695:1991 entitled "Glass—Resistance to attack by a boiling aqueous solution of mixed alkali—Method of test and classification"; and ISO 720:1985 entitled "Glass—Hydrolytic resistance of glass grains at 121 degrees C.—Method of test and classification." The chemical durability of the glass may also be assessed according to ISO 719:1985 "Glass—Hydrolytic resistance of glass grains at 98 degrees C.—Method of test and classification," in addition to the above referenced standards. The ISO 719 standard is a less rigorous version of the ISO 720 standard and, as such, it is believed that a glass which meets a specified classification of the ISO 720 standard will also meet the corresponding classification of the ISO 719 standard. The classifications associated with each standard are described in further detail herein.

Glass Compositions

Reference will now be made in detail to various embodiments of pharmaceutical containers formed, at least in part, of glass compositions which exhibit improved chemical and mechanical durability and, in particular, improved resistance to delamination. The glass compositions may also be chemically strengthened thereby imparting increased mechanical durability to the glass. The glass compositions described herein generally comprise silica (SiO_2), alumina (Al_2O_3),

alkaline earth oxides (such as MgO and/or CaO), and alkali oxides (such as Na_2O and/or K_2O) in amounts which impart chemical durability to the glass composition. Moreover, the alkali oxides present in the glass compositions facilitate chemically strengthening the glass compositions by ion exchange. Various embodiments of the glass compositions will be described herein and further illustrated with reference to specific examples.

The glass compositions described herein are alkali aluminosilicate glass compositions which generally include a combination of SiO_2, Al_2O_3, at least one alkaline earth oxide, and one or more alkali oxides, such as Na_2O and/or K_2O. In some embodiments, the glass compositions may be free from boron and compounds containing boron. The combination of these components enables a glass composition which is resistant to chemical degradation and is also suitable for chemical strengthening by ion exchange. In some embodiments the glass compositions may further comprise minor amounts of one or more additional oxides such as, for example, SnO_2, ZrO_2, ZnO, TiO_2, As_2O_3 or the like. These components may be added as fining agents and/or to further enhance the chemical durability of the glass composition.

In the embodiments of the glass compositions described herein SiO_2 is the largest constituent of the composition and, as such, is the primary constituent of the resulting glass network. SiO_2 enhances the chemical durability of the glass and, in particular, the resistance of the glass composition to decomposition in acid and the resistance of the glass composition to decomposition in water. Accordingly, a high SiO_2 concentration is generally desired. However, if the content of SiO_2 is too high, the formability of the glass may be diminished as higher concentrations of SiO_2 increase the difficulty of melting the glass which, in turn, adversely impacts the formability of the glass. In the embodiments described herein, the glass composition generally comprises SiO_2 in an amount greater than or equal to 67 mol. % and less than or equal to about 80 mol. % or even less than or equal to 78 mol. %. In some embodiments, the amount of SiO_2 in the glass composition may be greater than about 68 mol. %, greater than about 69 mol. % or even greater than about 70 mol. %. In some other embodiments, the amount of SiO_2 in the glass composition may be greater than 72 mol. %, greater than 73 mol. % or even greater than 74 mol. %. For example, in some embodiments, the glass composition may include from about 68 mol. % to about 80 mol. % or even to about 78 mol. % SiO_2. In some other embodiments the glass composition may include from about 69 mol. % to about 80 mol. % or even to about 78 mol. % SiO_2. In some other embodiments the glass composition may include from about 70 mol. % to about 80 mol. % or even to about 78 mol. % SiO_2. In still other embodiments, the glass composition comprises SiO_2 in an amount greater than or equal to 70 mol. % and less than or equal to 78 mol. %. In some embodiments, SiO_2 may be present in the glass composition in an amount from about 72 mol. % to about 78 mol. %. In some other embodiments, SiO_2 may be present in the glass composition in an amount from about 73 mol. % to about 78 mol. %. In other embodiments, SiO_2 may be present in the glass composition in an amount from about 74 mol. % to about 78 mol. %. In still other embodiments, SiO_2 may be present in the glass composition in an amount from about 70 mol. % to about 76 mol. %.

The glass compositions described herein further include Al_2O_3. Al_2O_3, in conjunction with alkali oxides present in the glass compositions such as Na_2O or the like, improves the susceptibility of the glass to ion exchange strengthening. In the embodiments described herein, Al_2O_3 is present in the glass compositions in X mol. % while the alkali oxides are present in the glass composition in Y mol. %. The ratio Y:X in the glass compositions described herein is greater than 1 in order to facilitate the aforementioned susceptibility to ion exchange strengthening. Specifically, the diffusion coefficient or diffusivity D of the glass composition relates to the rate at which alkali ions penetrate into the glass surface during ion exchange. Glasses which have a ratio Y:X greater than about 0.9 or even greater than about 1 have a greater diffusivity than glasses which have a ratio Y:X less than 0.9. Glasses in which the alkali ions have a greater diffusivity can obtain a greater depth of layer for a given ion exchange time and ion exchange temperature than glasses in which the alkali ions have a lower diffusivity. Moreover, as the ratio of Y:X increases, the strain point, anneal point, and softening point of the glass decrease, such that the glass is more readily formable. In addition, for a given ion exchange time and ion exchange temperature, it has been found that compressive stresses induced in glasses which have a ratio Y:X greater than about 0.9 and less than or equal to 2 are generally greater than those generated in glasses in which the ratio Y:X is less than 0.9 or greater than 2. Accordingly, in some embodiments, the ratio of Y:X is greater than 0.9 or even greater than 1. In some embodiments, the ratio of Y:X is greater than 0.9, or even greater than 1, and less than or equal to about 2. In still other embodiments, the ratio of Y:X may be greater than or equal to about 1.3 and less than or equal to about 2.0 in order to maximize the amount of compressive stress induced in the glass for a specified ion exchange time and a specified ion exchange temperature.

However, if the amount of Al_2O_3 in the glass composition is too high, the resistance of the glass composition to acid attack is diminished. Accordingly, the glass compositions described herein generally include Al_2O_3 in an amount greater than or equal to about 2 mol. % and less than or equal to about 10 mol. %. In some embodiments, the amount of Al_2O_3 in the glass composition is greater than or equal to about 4 mol. % and less than or equal to about 8 mol. %. In some other embodiments, the amount of Al_2O_3 in the glass composition is greater than or equal to about 5 mol. % to less than or equal to about 7 mol. %. In some other embodiments, the amount of Al_2O_3 in the glass composition is greater than or equal to about 6 mol. % to less than or equal to about 8 mol. %. In still other embodiments, the amount of Al_2O_3 in the glass composition is greater than or equal to about 5 mol. % to less than or equal to about 6 mol. %.

The glass compositions also include one or more alkali oxides such as Na_2O and/or K_2O. The alkali oxides facilitate the ion exchangeability of the glass composition and, as such, facilitate chemically strengthening the glass. The alkali oxide may include one or more of Na_2O and K_2O. The alkali oxides are generally present in the glass composition in a total concentration of Y mol. %. In some embodiments described herein, Y may be greater than about 2 mol. % and less than or equal to about 18 mol. %. In some other embodiments, Y may be greater than about 8 mol. %, greater than about 9 mol. %, greater than about 10 mol. % or even greater than about 11 mol. %. For example, in some embodiments described herein Y is greater than or equal to about 8 mol. % and less than or equal to about 18 mol. %. In still other embodiments, Y may be greater than or equal to about 9 mol. % and less than or equal to about 14 mol. %.

The ion exchangeability of the glass composition is primarily imparted to the glass composition by the amount of the alkali oxide Na_2O initially present in the glass composition prior to ion exchange. Accordingly, in the

embodiments of the glass compositions described herein, the alkali oxide present in the glass composition includes at least Na_2O. Specifically, in order to achieve the desired compressive strength and depth of layer in the glass composition upon ion exchange strengthening, the glass compositions include Na_2O in an amount from about 2 mol. % to about 15 mol. % based on the molecular weight of the glass composition. In some embodiments the glass composition includes at least about 8 mol. % of Na_2O based on the molecular weight of the glass composition. For example, the concentration of Na_2O may be greater than 9 mol. %, greater than 10 mol. % or even greater than 11 mol. %. In some embodiments, the concentration of Na_2O may be greater than or equal to 9 mol. % or even greater than or equal to 10 mol. %. For example, in some embodiments the glass composition may include Na_2O in an amount greater than or equal to about 9 mol. % and less than or equal to about 15 mol. % or even greater than or equal to about 9 mol. % and less than or equal to 13 mol. %.

As noted above, the alkali oxide in the glass composition may further include K_2O. The amount of K_2O present in the glass composition also relates to the ion exchangeability of the glass composition. Specifically, as the amount of K_2O present in the glass composition increases, the compressive stress obtainable through ion exchange decreases as a result of the exchange of potassium and sodium ions. Accordingly, it is desirable to limit the amount of K_2O present in the glass composition. In some embodiments, the amount of K_2O is greater than or equal to 0 mol. % and less than or equal to 3 mol. %. In some embodiments, the amount of K_2O is less or equal to 2 mol. % or even less than or equal to 1.0 mol. %. In embodiments where the glass composition includes K_2O, the K_2O may be present in a concentration greater than or equal to about 0.01 mol. % and less than or equal to about 3.0 mol. % or even greater than or equal to about 0.01 mol. % and less than or equal to about 2.0 mol. %. In some embodiments, the amount of K_2O present in the glass composition is greater than or equal to about 0.01 mol. % and less than or equal to about 1.0 mol. %. Accordingly, it should be understood that K_2O need not be present in the glass composition. However, when K_2O is included in the glass composition, the amount of K_2O is generally less than about 3 mol. % based on the molecular weight of the glass composition.

The alkaline earth oxides present in the composition improve the meltability of the glass batch materials and increase the chemical durability of the glass composition. In the glass compositions described herein, the total mol. % of alkaline earth oxides present in the glass compositions is generally less than the total mol. % of alkali oxides present in the glass compositions in order to improve the ion exchangeability of the glass composition. In the embodiments described herein, the glass compositions generally include from about 3 mol. % to about 13 mol. % of alkaline earth oxide. In some of these embodiments, the amount of alkaline earth oxide in the glass composition may be from about 4 mol. % to about 8 mol. % or even from about 4 mol. % to about 7 mol. %.

The alkaline earth oxide in the glass composition may include MgO, CaO, SrO, BaO or combinations thereof. In some embodiments, the alkaline earth oxide includes MgO, CaO or combinations thereof. For example, in the embodiments described herein the alkaline earth oxide includes MgO. MgO is present in the glass composition in an amount which is greater than or equal to about 3 mol. % and less than or equal to about 8 mol. % MgO. In some embodiments, MgO may be present in the glass composition in an amount which is greater than or equal to about 3 mol. % and less than or equal to about 7 mol. % or even greater than or equal to 4 mol. % and less than or equal to about 7 mol. % by molecular weight of the glass composition.

In some embodiments, the alkaline earth oxide may further include CaO. In these embodiments CaO is present in the glass composition in an amount from about 0 mol. % to less than or equal to 6 mol. % by molecular weight of the glass composition. For example, the amount of CaO present in the glass composition may be less than or equal to 5 mol. %, less than or equal to 4 mol. %, less than or equal to 3 mol. %, or even less than or equal to 2 mol. %. In some of these embodiments, CaO may be present in the glass composition in an amount greater than or equal to about 0.1 mol. % and less than or equal to about 1.0 mol. %. For example, CaO may be present in the glass composition in an amount greater than or equal to about 0.2 mol. % and less than or equal to about 0.7 mol. % or even in an amount greater than or equal to about 0.3 mol. % and less than or equal to about 0.6 mol. %.

In the embodiments described herein, the glass compositions are generally rich in MgO, (i.e., the concentration of MgO in the glass composition is greater than the concentration of the other alkaline earth oxides in the glass composition including, without limitation, CaO). Forming the glass composition such that the glass composition is MgO-rich improves the hydrolytic resistance of the resultant glass, particularly following ion exchange strengthening. Moreover, glass compositions which are MgO-rich generally exhibit improved ion exchange performance relative to glass compositions which are rich in other alkaline earth oxides. Specifically, glasses formed from MgO-rich glass compositions generally have a greater diffusivity than glass compositions which are rich in other alkaline earth oxides, such as CaO. The greater diffusivity enables the formation of a deeper depth of layer in the glass. MgO-rich glass compositions also enable a higher compressive stress to be achieved in the surface of the glass compared to glass compositions which are rich in other alkaline earth oxides such as CaO. In addition, it is generally understood that as the ion exchange process proceeds and alkali ions penetrate more deeply into the glass, the maximum compressive stress achieved at the surface of the glass may decrease with time. However, glasses formed from glass compositions which are MgO-rich exhibit a lower reduction in compressive stress than glasses formed from glass compositions that are CaO-rich or rich in other alkaline earth oxides (i.e., glasses which are MgO-poor). Thus, MgO-rich glass compositions enable glasses which have higher compressive stress at the surface and greater depths of layer than glasses which are rich in other alkaline earth oxides.

In order to fully realize the benefits of MgO in the glass compositions described herein, it has been determined that the ratio of the concentration of CaO to the sum of the concentration of CaO and the concentration of MgO in mol. % (i.e., (CaO/(CaO+MgO)) should be minimized. Specifically, it has been determined that (CaO/(CaO+MgO)) should be less than or equal to 0.5. In some embodiments (CaO/(CaO+MgO)) is less than or equal to 0.3 or even less than or equal to 0.2. In some other embodiments (CaO/(CaO+MgO)) may even be less than or equal to 0.1.

Boron oxide (B_2O_3) is a flux which may be added to glass compositions to reduce the viscosity at a given temperature (e.g., the strain, anneal and softening temperatures) thereby improving the formability of the glass. However, it has been found that additions of boron significantly decrease the diffusivity of sodium and potassium ions in the glass com-

position which, in turn, adversely impacts the ion exchange performance of the resultant glass. In particular, it has been found that additions of boron significantly increase the time required to achieve a given depth of layer relative to glass compositions which are boron free. Accordingly, in some embodiments described herein, the amount of boron added to the glass composition is minimized in order to improve the ion exchange performance of the glass composition.

For example, it has been determined that the impact of boron on the ion exchange performance of a glass composition can be mitigated by controlling the ratio of the concentration of B_2O_3 to the difference between the total concentration of the alkali oxides (i.e., R_2O, where R is the alkali metals) and alumina (i.e., B_2O_3 (mol. %)/(R_2O (mol. %)-Al_2O_3 (mol. %)). In particular, it has been determined that when the ratio of B_2O_3/(R_2O—Al_2O_3) is greater than or equal to about 0 and less than about 0.3 or even less than about 0.2, the diffusivities of alkali oxides in the glass compositions are not diminished and, as such, the ion exchange performance of the glass composition is maintained. Accordingly, in some embodiments, the ratio of B_2O_3/(R_2O—Al_2O_3) is greater than 0 and less than or equal to 0.3. In some of these embodiments, the ratio of B_2O_3/(R_2O—Al_2O_3) is greater than 0 and less than or equal to 0.2. In some embodiments, the ratio of B_2O_3/(R_2O—Al_2O_3) is greater than 0 and less than or equal to 0.15 or even less than or equal to 0.1. In some other embodiments, the ratio of B_2O_3/(R_2O—Al_2O_3) may be greater than 0 and less than or equal to 0.05. Maintaining the ratio B_2O_3/(R_2O—Al_2O_3) to be less than or equal to 0.3 or even less than or equal to 0.2 permits the inclusion of B_2O_3 to lower the strain point, anneal point and softening point of the glass composition without the B_2O_3 adversely impacting the ion exchange performance of the glass.

In the embodiments described herein, the concentration of B_2O_3 in the glass composition is generally less than or equal to about 4 mol. %, less than or equal to about 3 mol. %, less than or equal to about 2 mol. %, or even less than or equal to 1 mol. %. For example, in embodiments where B_2O_3 is present in the glass composition, the concentration of B_2O_3 may be greater than about 0.01 mol. % and less than or equal to 4 mol. %. In some of these embodiments, the concentration of B_2O_3 may be greater than about 0.01 mol. % and less than or equal to 3 mol. %. In some embodiments, the B_2O_3 may be present in an amount greater than or equal to about 0.01 mol. % and less than or equal to 2 mol. %, or even less than or equal to 1.5 mol. %. Alternatively, the B_2O_3 may be present in an amount greater than or equal to about 1 mol. % and less than or equal to 4 mol. %, greater than or equal to about 1 mol. % and less than or equal to 3 mol. % or even greater than or equal to about 1 mol. % and less than or equal to 2 mol. %. In some of these embodiments, the concentration of B_2O_3 may be greater than or equal to about 0.1 mol. % and less than or equal to 1.0 mol. %.

While in some embodiments the concentration of B_2O_3 in the glass composition is minimized to improve the forming properties of the glass without detracting from the ion exchange performance of the glass, in some other embodiments the glass compositions are free from boron and compounds of boron such as B_2O_3. Specifically, it has been determined that forming the glass composition without boron or compounds of boron improves the ion exchangeability of the glass compositions by reducing the process time and/or temperature required to achieve a specific value of compressive stress and/or depth of layer.

In some embodiments of the glass compositions described herein, the glass compositions are free from phosphorous and compounds containing phosphorous including, without limitation, P_2O_5. Specifically, it has been determined that formulating the glass composition without phosphorous or compounds of phosphorous increases the chemical durability of the glass composition.

In addition to the SiO_2, Al_2O_3, alkali oxides and alkaline earth oxides, the glass compositions described herein may optionally further comprise one or more fining agents such as, for example, SnO_2, As_2O_3, and/or Cl^- (from NaCl or the like). When a fining agent is present in the glass composition, the fining agent may be present in an amount less than or equal to about 1 mol. % or even less than or equal to about 0.4 mol. %. For example, in some embodiments the glass composition may include SnO_2 as a fining agent. In these embodiments SnO_2 may be present in the glass composition in an amount greater than about 0 mol. % and less than or equal to about 1 mol. % or even an amount greater than or equal to about 0.01 mol. % and less than or equal to about 0.30 mol. %.

Moreover, the glass compositions described herein may comprise one or more additional metal oxides to further improve the chemical durability of the glass composition. For example, the glass composition may further include ZnO, TiO_2, or ZrO_2, each of which further improves the resistance of the glass composition to chemical attack. In these embodiments, the additional metal oxide may be present in an amount which is greater than or equal to about 0 mol. % and less than or equal to about 2 mol. %. For example, when the additional metal oxide is ZnO, the ZnO may be present in an amount greater than or equal to 1 mol. % and less than or equal to about 2 mol. %. When the additional metal oxide is ZrO_2 or TiO_2, the ZrO_2 or TiO_2 may be present in an amount less than or equal to about 1 mol. %.

Based on the foregoing, it should be understood that, in a first exemplary embodiment, a glass composition may include: SiO_2 in a concentration greater than about 70 mol. % and Y mol. % alkali oxide. The alkali oxide may include Na_2O in an amount greater than about 8 mol. %. The glass composition may be free of boron and compounds of boron. The concentration of SiO_2 in this glass composition may be greater than or equal to about 72 mol. %, greater than 73 mol. % or even greater than 74 mol. %. The glass composition of this first exemplary embodiment may be free from phosphorous and compounds of phosphorous. The glass composition may also include X mol. % Al_2O_3. When Al_2O_3 is included, the ratio of Y:X may be greater than 1. The concentration of Al_2O_3 may be greater than or equal to about 2 mol. % and less than or equal to about 10 mol. %.

The glass composition of this first exemplary embodiment may also include alkaline earth oxide in an amount from about 3 mol. % to about 13 mol. %. The alkaline earth oxide may include MgO and CaO. The CaO may be present in an amount greater than or equal to about 0.1 mol. % and less than or equal to about 1.0 mol. %. A ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) may be less than or equal to 0.5.

In a second exemplary embodiment, a glass composition may include: greater than about 68 mol. % SiO_2; X mol. % Al_2O_3; Y mol. % alkali oxide; and B_2O_3. The alkali oxide may include Na_2O in an amount greater than about 8 mol %. A ratio (B_2O_3 (mol. %)/(Y mol. %–X mol. %) may be greater than 0 and less than 0.3. The concentration of SiO_2 in this glass composition may be greater than or equal to about 72 mol. %, greater than 73 mol. % or even greater than 74 mol. %. The concentration of Al_2O_3 may be greater than or equal to about 2 mol. % and less than or equal to about

10 mol. %. In this second exemplary embodiment, the ratio of Y:X may be greater than 1. When the ratio of Y:X is greater than 1, an upper bound of the ratio of Y:X may be less than or equal to 2. The glass composition of this first exemplary embodiment may be free from phosphorous and compounds of phosphorous.

The glass composition of this second exemplary embodiment may also include alkaline earth oxide. The alkaline earth oxide may include MgO and CaO. The CaO may be present in an amount greater than or equal to about 0.1 mol. % and less than or equal to about 1.0 mol. %. A ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) may be less than or equal to 0.5.

The concentration of B_2O_3 in this second exemplary embodiment may be greater than or equal to about 0.01 mol. % and less than or equal to about 4 mol. %.

In a third exemplary embodiment, a glass article may have a type HgB1 hydrolytic resistance according to ISO 719. The glass article may include greater than about 8 mol. % Na_2O and less than about 4 mol. % B_2O_3. The glass article may further comprise X mol. % Al_2O_3 and Y mol. % alkali oxide. The ratio (B_2O_3 (mol. %)/(Y mol. %−X mol. %) may be greater than 0 and less than 0.3. The glass article of this third exemplary embodiment may further include a compressive stress layer having a surface compressive stress greater than or equal to about 250 MPa. The glass article may also have at least a class S3 acid resistance according to DIN 12116; at least a class A2 base resistance according to ISO 695; and a type HgAl hydrolytic resistance according to ISO 720.

In a fourth exemplary embodiment, a glass pharmaceutical package may include SiO_2 in an amount greater than about 70 mol. %; X mol. % Al_2O_3; and Y mol. % alkali oxide. The alkali oxide may include Na_2O in an amount greater than about 8 mol. %. A ratio of a concentration of B_2O_3 (mol. %) in the glass pharmaceutical package to (Y mol. %−X mol. %) may be less than 0.3. The glass pharmaceutical package may also have a type HGB1 hydrolytic resistance according to ISO 719. The concentration of SiO_2 in the glass pharmaceutical package of this fourth exemplary embodiment may be greater than or equal to 72 mol. % and less than or equal to about 78 mol. % or even greater than 74 mol. % and less than or equal to about 78 mol. %. The concentration of Al_2O_3 in the glass pharmaceutical may be greater than or equal to about 4 mol. % and less than or equal to about 8 mol. %. A ratio of Y:X may be greater than 1 and less than 2.

The glass pharmaceutical package of this fourth exemplary embodiment may also include alkaline earth oxide in an amount from about 4 mol. % to about 8 mol. %. The alkaline earth oxide may include MgO and CaO. The CaO may be present in an amount greater than or equal to about 0.2 mol. % and less than or equal to about 0.7 mol. %. A ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) may be less than or equal to 0.5. The glass pharmaceutical package of this fourth exemplary embodiment may have a type HGA1 hydrolytic resistance according to ISO 720.

In a fifth exemplary embodiment, a glass composition may include from about 70 mol. % to about 80 mol. % SiO_2; from about 3 mol. % to about 13 mol. % alkaline earth oxide; X mol. % Al_2O_3; and Y mol. % alkali oxide. The alkali oxide may include Na_2O in an amount greater than about 8 mol. %. A ratio of Y:X may be greater than 1. The glass composition may be free of boron and compounds of boron.

In a sixth exemplary embodiment, a glass composition may include from about 68 mol. % to about 80 mol. % SiO_2; from about 3 mol. % to about 13 mol. % alkaline earth oxide; X mol. % Al_2O_3; and Y mol. % alkali oxide. The alkali oxide may include Na_2O in an amount greater than about 8 mol. %. The glass composition of this sixth exemplary embodiment may also include B_2O_3. A ratio (B_2O_3 (mol. %)/(Y mol. %−X mol. %) may be greater than 0 and less than 0.3. A ratio of Y:X may be greater than 1.

In a seventh exemplary embodiment, a glass composition may include from about 70 mol. % to about 80 mol. % SiO_2; from about 3 mol. % to about 13 mol. % alkaline earth oxide; X mol. % Al_2O_3; and Y mol. % alkali oxide. The amount of Al_2O_3 in the glass composition may be greater than or equal to about 2 mol. % and less than or equal to about 10 mol. %. The alkaline earth oxide may include CaO in an amount greater than or equal to about 0.1 mol. % and less than or equal to about 1.0 mol. %. The alkali oxide may include from about 0.01 mol. % to about 1.0 mol. % K_2O. A ratio of Y:X may be greater than 1. The glass composition may be free of boron and compounds of boron. The glass composition may be amenable to strengthening by ion exchange.

In a seventh exemplary embodiment, a glass composition may include SiO_2 in an amount greater than about 70 mol. % and less than or equal to about 80 mol. %; X mol. % Al_2O_3; and Y mol. % alkali oxide. The alkali oxide may include Na_2O in an amount greater than about 8 mol. %. A ratio of a concentration of B_2O_3 (mol. %) in the glass pharmaceutical package to (Y mol. %−X mol. %) may be less than 0.3. A ratio of Y:X may be greater than 1.

In an eighth exemplary embodiment, a glass composition may include from about 72 mol. % to about 78 mol. % SiO_2; from about 4 mol. % to about 8 mol. % alkaline earth oxide; X mol. % Al_2O_3, wherein X is greater than or equal to about 4 mol. % and less than or equal to about 8 mol. %; and Y mol. % alkali oxide, wherein the alkali oxide comprises Na_2O in an amount greater than or equal to about 9 mol. % and less than or equal to about 15 mol. %. A ratio of a concentration of B_2O_3 (mol. %) in the glass pharmaceutical package to (Y mol. %−X mol. %) is less than 0.3. A ratio of Y:X may be greater than 1.

In a ninth exemplary embodiment, a pharmaceutical package for containing a pharmaceutical composition may include from about 70 mol. % to about 78 mol. % SiO_2; from about 3 mol. % to about 13 mol. % alkaline earth oxide; X mol. % Al_2O_3, wherein X is greater than or equal to 2 mol. % and less than or equal to about 10 mol. %; and Y mol. % alkali oxide, wherein the alkali oxide comprises Na_2O in an amount greater than about 8 mol. %. The alkaline earth oxide may include CaO in an amount less than or equal to about 6.0 mol. %. A ratio of Y:X may be greater than about 1. The package may be free of boron and compounds of boron and may include a compressive stress layer with a compressive stress greater than or equal to about 250 MPa and a depth of layer greater than or equal to about 10 μm.

In a tenth exemplary embodiment, a glass article may be formed from a glass composition comprising from about 70 mol. % to about 78 mol. % SiO_2; alkaline earth oxide, wherein the alkaline earth oxide comprises MgO and CaO and a ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) is less than or equal to 0.5; X mol. % Al_2O_3, wherein X is from about 2 mol. % to about 10 mol. %; and Y mol. % alkali oxide, wherein the alkali oxide comprises Na_2O in an amount greater than about 8 mol. % and a ratio of Y:X is greater than 1. The glass article may be ion exchange strengthened with a compressive stress greater than or equal to 250 MPa and a depth of layer greater than or equal to 10 μm. The glass article may have a type HgAl hydrolytic resistance according to ISO 720.

As noted above, the presence of alkali oxides in the glass composition facilitates chemically strengthening the glass by ion exchange. Specifically, alkali ions, such as potassium ions, sodium ions and the like, are sufficiently mobile in the glass to facilitate ion exchange. In some embodiments, the glass composition is ion exchangeable to form a compressive stress layer having a depth of layer greater than or equal to 10 μm. In some embodiments, the depth of layer may be greater than or equal to about 25 μm or even greater than or equal to about 50 μm. In some other embodiments, the depth of the layer may be greater than or equal to 75 μm or even greater than or equal to 100 μm. In still other embodiments, the depth of layer may be greater than or equal to 10 μm and less than or equal to about 100 μm. The associated surface compressive stress may be greater than or equal to about 250 MPa, greater than or equal to 300 MPa or even greater than or equal to about 350 MPa after the glass composition is treated in a salt bath of 100% molten KNO_3 at a temperature of 350° C. to 500° C. for a time period of less than about 30 hours or even about less than 20 hours.

The glass articles formed from the glass compositions described herein may have a hydrolytic resistance of HGB2 or even HGB1 under ISO 719 and/or a hydrolytic resistance of HGA2 or even HGA1 under ISO 720 (as described further herein) in addition to having improved mechanical characteristics due to ion exchange strengthening. In some embodiments described herein the glass articles may have compressive stresses which extend from the surface into the glass article to a depth of layer greater than or equal to 10 μm, greater than or equal to 15 μm, greater than or equal to 20 μm, greater than or equal to 25 μm, greater than or equal to 30 μm or even greater than or equal to 35 μm. In some embodiments, the depth of layer may be greater than or equal to 40 μm or even greater than or equal to 50 μm. The surface compressive stress of the glass article may be greater than or equal to 150 MPa, greater than or equal to 200 MPa, greater than or equal to 250 MPa, greater than or equal to 350 MPa, or even greater than or equal to 400 MPa.

In one embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 150 MPa and a depth of layer greater than or equal to 10 μm, 15 μm, 20 μm, 25 μm, 30 μm, 35 μm, 40 μm, 45 μm or 50 μm. In a particular embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 150 MPa and a depth of layer greater than or equal to 10 μm. In a particular embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 150 MPa and a depth of layer greater than or equal to 25 μm. In a particular embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 150 MPa and a depth of layer greater than or equal to 30 μm.

In one embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 300 MPa and a depth of layer greater than or equal to 10 μm, 15 μm, 20 μm, 25 μm, 30 μm, 35 μm, 40 μm, 45 μm or 50 μm. In a particular embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 300 MPa and a depth of layer greater than or equal to 25 μm. In yet another embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 300 MPa and a depth of layer greater than or equal to 30 μm. In yet another embodiment, the glass pharmaceutical container has a compressive stress greater than or equal to 300 MPa and a depth of layer greater than or equal to 35 μm.

The glass compositions described herein facilitate achieving the aforementioned depths of layer and surface compressive stresses more rapidly and/or at lower temperatures than conventional glass compositions due to the enhanced alkali ion diffusivity of the glass compositions as described hereinabove. For example, the depths of layer (i.e., greater than or equal to 25 μm) and the compressive stresses (i.e., greater than or equal to 250 MPa) may be achieved by ion exchanging the glass article in a molten salt bath of 100% KNO_3 (or a mixed salt bath of KNO_3 and $NaNO_3$) for a time period of less than or equal to 5 hours or even less than or equal to 4.5 hours. In some embodiments, these depths of layer and compressive stresses may be achieved by ion exchanging the glass article in a molten salt bath of 100% KNO_3 (or a mixed salt bath of KNO_3 and $NaNO_3$) for a time period of less than or equal to 4 hours or even less than or equal to 3.5 hours. Moreover, these depths of layers and compressive stresses may be achieved by ion exchanging the glass articles in a molten salt bath of 100% KNO3 (or a mixed salt bath of KNO_3 and $NaNO_3$) at a temperature less than or equal to 500° C. or even less than or equal to 450° C. In some embodiments, these depths of layers and compressive stresses may be achieved by ion exchanging the glass articles in a molten salt bath of 100% KNO3 (or a mixed salt bath of KNO_3 and $NaNO_3$) at a temperature less than or equal to 400° C. or even less than or equal to 350° C.

These improved ion exchange characteristics can be achieved when the glass composition has a threshold diffusivity of greater than about 16 $\mu m^2/hr$ or even greater than or equal to 20 $\mu m^2/hr$ at 450° C. In some embodiments, the threshold diffusivity may be greater than or equal to about 25 m^2/hr or even 30 $\mu m^2/hr$ at 450° C. In some other embodiments, the threshold diffusivity may be greater than or equal to about 35 $\mu m^2/hr$ or even 40 $\mu m^2/hr$ at 450° C. In still other embodiments, the threshold diffusivity may be greater than or equal to about 45 $\mu m^2/hr$ or even 50 $\mu m^2/hr$ at 450° C.

The glass compositions described herein may generally have a strain point greater than or equal to about 525° C. and less than or equal to about 650° C. The glasses may also have an anneal point greater than or equal to about 560° C. and less than or equal to about 725° C. and a softening point greater than or equal to about 750° C. and less than or equal to about 960° C.

In the embodiments described herein the glass compositions have a CTE of less than about $70\times10^{-7}\ K^{-1}$ or even less than about $60\times10^{-7}\ K^{-1}$. These lower CTE values improve the survivability of the glass to thermal cycling or thermal stress conditions relative to glass compositions with higher CTEs.

Further, as noted hereinabove, the glass compositions are chemically durable and resistant to degradation as determined by the DIN 12116 standard, the ISO 695 standard, and the ISO 720 standard.

Specifically, the DIN 12116 standard is a measure of the resistance of the glass to decomposition when placed in an acidic solution. In brief, the DIN 12116 standard utilizes a polished glass sample of a known surface area which is weighed and then positioned in contact with a proportional amount of boiling 6M hydrochloric acid for 6 hours. The sample is then removed from the solution, dried and weighed again. The glass mass lost during exposure to the acidic solution is a measure of the acid durability of the sample with smaller numbers indicative of greater durability. The results of the test are reported in units of half-mass per surface area, specifically mg/dm^2. The DIN 12116 standard is broken into individual classes. Class S1 indicates weight losses of up to 0.7 mg/dm^2; Class S2 indicates weight losses from 0.7 mg/dm^2 up to 1.5 mg/dm^2; Class S3 indi-

cates weight losses from 1.5 mg/dm^2 up to 15 mg/dm^2; and Class S4 indicates weight losses of more than 15 mg/dm^2.

The ISO 695 standard is a measure of the resistance of the glass to decomposition when placed in a basic solution. In brief, the ISO 695 standard utilizes a polished glass sample which is weighed and then placed in a solution of boiling 1M NaOH+0.5M Na_2CO_3 for 3 hours. The sample is then removed from the solution, dried and weighed again. The glass mass lost during exposure to the basic solution is a measure of the base durability of the sample with smaller numbers indicative of greater durability. As with the DIN 12116 standard, the results of the ISO 695 standard are reported in units of mass per surface area, specifically mg/dm^2. The ISO 695 standard is broken into individual classes. Class A1 indicates weight losses of up to 75 mg/dm^2; Class A2 indicates weight losses from 75 mg/dm^2 up to 175 mg/dm^2; and Class A3 indicates weight losses of more than 175 mg/dm^2.

The ISO 720 standard is a measure of the resistance of the glass to degradation in purified, CO_2-free water. In brief, the ISO 720 standard protocol utilizes crushed glass grains which are placed in contact with the purified, CO_2-free water under autoclave conditions (121° C., 2 atm) for 30 minutes. The solution is then titrated colorimetrically with dilute HCl to neutral pH. The amount of HCl required to titrate to a neutral solution is then converted to an equivalent of Na_2O extracted from the glass and reported in μg Na_2O per weight of glass with smaller values indicative of greater durability. The ISO 720 standard is broken into individual types. Type HGA1 is indicative of up to 62 μg extracted equivalent of Na_2O per gram of glass tested; Type HGA2 is indicative of more than 62 μg and up to 527 μg extracted equivalent of Na_2O per gram of glass tested; and Type HGA3 is indicative of more than 527 μg and up to 930 μg extracted equivalent of Na_2O per gram of glass tested.

The ISO 719 standard is a measure of the resistance of the glass to degradation in purified, CO_2-free water. In brief, the ISO 719 standard protocol utilizes crushed glass grains which are placed in contact with the purified, CO_2-free water at a temperature of 98° C. at 1 atmosphere for 30 minutes. The solution is then titrated colorimetrically with dilute HCl to neutral pH. The amount of HCl required to titrate to a neutral solution is then converted to an equivalent of Na_2O extracted from the glass and reported in μg Na_2O per weight of glass with smaller values indicative of greater durability. The ISO 719 standard is broken into individual types. The ISO 719 standard is broken into individual types. Type HGB1 is indicative of up to 31 μg extracted equivalent of Na_2O; Type HGB2 is indicative of more than 31 μg and up to 62 μg extracted equivalent of Na_2O; Type HGB3 is indicative of more than 62 μg and up to 264 μg extracted equivalent of Na_2O; Type HGB4 is indicative of more than 264 μg and up to 620 μg extracted equivalent of Na_2O; and Type HGB5 is indicative of more than 620 μg and up to 1085 μg extracted equivalent of Na_2O. The glass compositions described herein have an ISO 719 hydrolytic resistance of type HGB2 or better with some embodiments having a type HGB1 hydrolytic resistance.

The glass compositions described herein have an acid resistance of at least class S3 according to DIN 12116 both before and after ion exchange strengthening with some embodiments having an acid resistance of at least class S2 or even class 51 following ion exchange strengthening. In some other embodiments, the glass compositions may have an acid resistance of at least class S2 both before and after ion exchange strengthening with some embodiments having an acid resistance of class 51 following ion exchange strengthening. Further, the glass compositions described herein have a base resistance according to ISO 695 of at least class A2 before and after ion exchange strengthening with some embodiments having a class A1 base resistance at least after ion exchange strengthening. The glass compositions described herein also have an ISO 720 type HGA2 hydrolytic resistance both before and after ion exchange strengthening with some embodiments having a type HGA1 hydrolytic resistance after ion exchange strengthening and some other embodiments having a type HGA1 hydrolytic resistance both before and after ion exchange strengthening. The glass compositions described herein have an ISO 719 hydrolytic resistance of type HGB2 or better with some embodiments having a type HGB1 hydrolytic resistance. It should be understood that, when referring to the above referenced classifications according to DIN 12116, ISO 695, ISO 720 and ISO 719, a glass composition or glass article which has "at least" a specified classification means that the performance of the glass composition is as good as or better than the specified classification. For example, a glass article which has a DIN 12116 acid resistance of "at least class S2" may have a DIN 12116 classification of either S1 or S2.

The glass compositions described herein are formed by mixing a batch of glass raw materials (e.g., powders of SiO_2, Al_2O_3, alkali oxides, alkaline earth oxides and the like) such that the batch of glass raw materials has the desired composition. Thereafter, the batch of glass raw materials is heated to form a molten glass composition which is subsequently cooled and solidified to form the glass composition. During solidification (i.e., when the glass composition is plastically deformable) the glass composition may be shaped using standard forming techniques to shape the glass composition into a desired final form. Alternatively, the glass article may be shaped into a stock form, such as a sheet, tube or the like, and subsequently reheated and formed into the desired final form.

In order to assess the long-term resistance of the glass container to delamination, an accelerated delamination test was utilized. The test is performed on glass containers after the containers have been ion-exchange strengthened. The test consisted of washing the glass container at room temperature for 1 minute and depyrogenating the container at about 320° C. for 1 hour. Thereafter a solution of 20 mM glycine with a pH of 10 in water is placed in the glass container to 80-90% fill, the glass container is closed, and rapidly heated to 100° C. and then heated from 100° C. to 121° C. at a ramp rate of 1 deg/min at a pressure of 2 atmospheres. The glass container and solution are held at this temperature for 60 minutes, cooled to room temperature at a rate of 0.5 deg./min and the heating cycle and hold are repeated. The glass container is then heated to 50° C. and held for two days for elevated temperature conditioning. After heating, the glass container is dropped from a distance of at least 18" onto a firm surface, such as a laminated tile floor, to dislodge any flakes or particles that are weakly adhered to the inner surface of the glass container.

Thereafter, the solution contained in the glass container is analyzed to determine the number of glass particles present per liter of solution. Specifically, the solution from the glass container is directly poured onto the center of a Millipore Isopore Membrane filter (Millipore #ATTP02500 held in an assembly with parts #AP1002500 and #M000025A0) attached to vacuum suction to draw the solution through the filter within 10-15 seconds. Particulate flakes are then counted by differential interference contrast microscopy (DIC) in the reflection mode as described in "Differential interference contrast (DIC) microscopy and modulation con-

trast microscopy" from Fundamentals of light microscopy and digital imaging. New York: Wiley-Liss, pp 153-168. The field of view is set to approximately 1.5 mm×1.5 mm and particles larger than 50 microns are counted manually. There are 9 such measurements made in the center of each filter membrane in a 3×3 pattern with no overlap between images. A minimum of 100 mL of solution is tested. As such, the solution from a plurality of small containers may be pooled to bring the total amount of solution to 100 mL. If the containers contain more than 10 mL of solution, the entire amount of solution from the container is examined for the presence of particles. For containers having a volume greater than 10 mL containers, the test is repeated for a trial of 10 containers formed from the same glass composition under the same processing conditions and the result of the particle count is averaged for the 10 containers to determine an average particle count. Alternatively, in the case of small containers, the test is repeated for a trial of 10 sets of 10 mL of solution, each of which is analyzed and the particle count averaged over the 10 sets to determine an average particle count. Averaging the particle count over multiple containers accounts for potential variations in the delamination behavior of individual containers.

It should be understood that the aforementioned test is used to identify particles which are shed from the interior wall(s) of the glass container due to delamination and not tramp particles present in the container from forming processes or particles which precipitate from the solution enclosed in the glass container as a result of reactions between the solution and the glass. Specifically, delamination particles may be differentiated from tramp glass particles due based on the aspect ratio of the particle (i.e., the ratio of the width of the particle to the thickness of the particle). Delamination produces particulate flakes or lamellae which are irregularly shaped and are typically >50 μm in diameter but often >200 μm. The thickness of the flakes is usually greater than about 100 nm and may be as large as about 1 μm. Thus, the minimum aspect ratio of the flakes is typically >50. The aspect ratio may be greater than 100 and sometimes greater than 1000. Particles resulting from delamination processes generally have an aspect ratio which is generally greater than about 50. In contrast, tramp glass particles will generally have a low aspect ratio which is less than about 3. Accordingly, particles resulting from delamination may be differentiated from tramp particles based on aspect ratio during observation with the microscope. Validation results can be accomplished by evaluating the heel region of the tested containers. Upon observation, evidence of skin corrosion/pitting/flake removal, as described in "Nondestructive Detection of Glass Vial Inner Surface Morphology with Differential Interference Contrast Microscopy" from Journal of Pharmaceutical Sciences 101(4), 2012, pages 1378-1384, is noted.

In the embodiments described herein, glass containers which average less than 3 glass particles with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered to have a delamination factor of 3. In the embodiments described herein, glass containers which average less than 2 glass particles with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered to have a delamination factor of 2. In the embodiments described herein, glass containers which average less than 1 glass particle with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered to have a delamination factor of 1. In the embodiments described herein, glass containers which have 0 glass particles with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered to have a delamination factor of 0. Accordingly, it should be understood that the lower the delamination factor, the better the resistance of the glass container to delamination. In the embodiments described herein, the glass containers have a delamination factor of 3 or lower (i.e., a delamination factor of 3, 2, 1 or 0).

Pharmaceutical Containers

In view of the chemical durability of the glass composition of the present invention, the glass compositions described herein are particularly well suited for use in designing pharmaceutical containers for storing, maintaining and/or delivering pharmaceutical compositions, such as liquids, solutions, powders, e.g., lyophilized powders, solids and the like. As used herein, the term "pharmaceutical container" refers to a composition designed to store, maintain and/or deliver a pharmaceutical composition. The pharmaceutical containers, as described herein, are formed, at least in part, of the delamination resistant glass compositions described above. Pharmaceutical containers of the present invention include, but are not limited to, Vacutainers™ cartridges, syringes, ampoules, bottles, flasks, phials, tubes, beakers, vials, injection pens or the like. In a particular embodiment, the pharmaceutical container is a vial. In a particular embodiment, the pharmaceutical container is an ampoule. In a particular embodiment, the pharmaceutical container is an injection pen. In a particular embodiment, the pharmaceutical container is a tube. In a particular embodiment, the pharmaceutical container is a bottle. In a particular embodiment, the pharmaceutical container is a syringe.

Moreover, the ability to chemically strengthen the glass compositions through ion exchange can be utilized to improve the mechanical durability of pharmaceutical containers formed from the glass composition. Accordingly, it should be understood that, in at least one embodiment, the glass compositions are incorporated in a pharmaceutical container in order to improve the chemical durability and/or the mechanical durability of the pharmaceutical container.

Pharmaceutical Compositions

In various embodiments, the pharmaceutical container further includes a pharmaceutical composition comprising an active pharmaceutical ingredient (API). As used herein, the term "pharmaceutical composition" refers to a composition comprising an active pharmaceutical ingredient to be delivered to a subject, for example, for therapeutic, prophylactic, diagnostic, preventative or prognostic effect. In certain embodiments, the pharmaceutical composition comprises the active pharmaceutical ingredient and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it may be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the active pharmaceutical agent.

As used herein, the term "active pharmaceutical ingredient" or "API" refers a substance in a pharmaceutical composition that provides a desired effect, for example, a therapeutic, prophylactic, diagnostic, preventative or prognostic effect. In various embodiments, the active pharmaceutical ingredient can be any of a variety of substances known in the art, for example, a small molecule, a polypeptide mimetic, a biologic, an antisense RNA, a small interfering RNA (siRNA), etc.

For example, in a particular embodiment, the active pharmaceutical ingredient may be a small molecule. As used herein, the term "small molecule" includes any chemical or other moiety, other than polypeptides and nucleic acids, that can act to affect biological processes. Small molecules can include any number of therapeutic agents presently known and used, or that can be synthesized from a library of such molecules for the purpose of screening for biological function(s). Small molecules are distinguished from macromolecules by size. The small molecules of the present invention usually have a molecular weight less than about 5,000 daltons (Da), preferably less than about 2,500 Da, more preferably less than 1,000 Da, most preferably less than about 500 Da.

Small molecules include, without limitation, organic compounds, peptidomimetics and conjugates thereof. As used herein, the term "organic compound" refers to any carbon-based compound other than macromolecules such as nucleic acids and polypeptides. In addition to carbon, organic compounds may contain calcium, chlorine, fluorine, copper, hydrogen, iron, potassium, nitrogen, oxygen, sulfur and other elements. An organic compound may be in an aromatic or aliphatic form. Non-limiting examples of organic compounds include acetones, alcohols, anilines, carbohydrates, monosaccharides, oligosaccharides, polysaccharides, amino acids, nucleosides, nucleotides, lipids, retinoids, steroids, proteoglycans, ketones, aldehydes, saturated, unsaturated and polyunsaturated fats, oils and waxes, alkenes, esters, ethers, thiols, sulfides, cyclic compounds, heterocyclic compounds, imidizoles, and phenols. An organic compound as used herein also includes nitrated organic compounds and halogenated (e.g., chlorinated) organic compounds.

In another embodiment, the active pharmaceutical ingredient may be a polypeptide mimetic ("peptidomimetic"). As used herein, the term "polypeptide mimetic" is a molecule that mimics the biological activity of a polypeptide, but that is not peptidic in chemical nature. While, in certain embodiments, a peptidomimetic is a molecule that contains no peptide bonds (that is, amide bonds between amino acids), the term peptidomimetic may include molecules that are not completely peptidic in character, such as pseudo-peptides, semi-peptides, and peptoids.

In other embodiments, the active pharmaceutical ingredient may be a biologic. As used herein, the term "biologic" includes products created by biologic processes instead of by chemical synthesis. Non-limiting examples of a "biologic" include proteins, antibodies, antibody like molecules, vaccines, blood, blood components, and partially purified products from tissues.

The terms "peptide," "polypeptide," and "protein" are used interchangeably herein. In the present invention, these terms mean a linked sequence of amino acids, which may be natural, synthetic, or a modification or combination of natural and synthetic. The term includes antibodies, antibody mimetics, domain antibodies, lipocalins, and targeted proteases. The term also includes vaccines containing a peptide or peptide fragment intended to raise antibodies against the peptide or peptide fragment.

"Antibody" as used herein includes an antibody of classes IgG, IgM, IgA, IgD, or IgE, or fragments or derivatives thereof, including Fab, F(ab')2, Fd, and single chain antibodies, diabodies, bispecific antibodies, and bifunctional antibodies. The antibody may be a monoclonal antibody, polyclonal antibody, affinity purified antibody, or mixtures thereof, which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom. The antibody may also be a chimeric antibody. The antibody may be derivatized by the attachment of one or more chemical, peptide, or polypeptide moieties known in the art. The antibody may be conjugated with a chemical moiety. The antibody may be a human or humanized antibody.

Other antibody-like molecules are also within the scope of the present invention. Such antibody-like molecules include, e.g., receptor traps (such as entanercept), antibody mimetics (such as adnectins, fibronectin based "addressable" therapeutic binding molecules from, e.g., Compound Therapeutics, Inc.), domain antibodies (the smallest functional fragment of a naturally occurring single-domain antibody (such as, e.g., nanobodies; see, e.g., Cortez-Retamozo et al., Cancer Res. 2004 Apr. 15; 64 (8):2853-7)).

Suitable antibody mimetics generally can be used as surrogates for the antibodies and antibody fragments described herein. Such antibody mimetics may be associated with advantageous properties (e.g., they may be water soluble, resistant to proteolysis, and/or be nonimmunogenic). For example, peptides comprising a synthetic beta-loop structure that mimics the second complementarity-determining region (CDR) of monoclonal antibodies have been proposed and generated. See, e.g., Saragovi et al., Science. Aug. 16, 1991; 253 (5021):792-5. Peptide antibody mimetics also have been generated by use of peptide mapping to determine "active" antigen recognition residues, molecular modeling, and a molecular dynamics trajectory analysis, so as to design a peptide mimic containing antigen contact residues from multiple CDRs. See, e.g., Cassett et al., Biochem Biophys Res Commun. Jul. 18, 2003; 307 (1):198-205. Additional discussion of related principles, methods, etc., that may be applicable in the context of this invention are provided in, e.g., Fassina, Immunomethods. October 1994; 5 (2):121-9.

In various embodiments, the active pharmaceutical ingredient may have any of a variety of activities selected from the group consisting of anti-rheumatics, anti-neoplastic, vaccines, anti-diabetics, haematologicals, muscle relaxant, immunostimulants, anti-coagulants, bone calcium regulators, sera and gammaglobulins, anti-fibrinolytics, MS therapies, anti-anaemics, cytostatics, interferons, anti-metabolites, radiopharmaceuticals, anti-psychotics, anti-bacterials, immunosuppressants, cytotoxic antibiotics, cerebral & peripheral vas otherapeutics, nootropics, CNS drugs, dermatologicals, angiotensin antagonists, anti-spasmodics, anti-cholinergics, interferons, anti-psoriasis agents, anti-hyperlipidaemics, cardiac therapies, alkylating agents, bronchodilators, anti-coagulants, anti-inflammatories, growth hormones, and diagnostic imaging agents.

In various embodiments, the pharmaceutical composition comprises GAMMAGARD LIQUID, an immune globulin composition (human), 10% solution, for intravenous and subcutaneous administration. In certain embodiments, the pharmaceutical composition comprises GAMMAGARD S/D, a human immune globulin composition for intravenous administration. In alternative embodiments, the pharmaceutical composition comprises GAMMAGARD S/D, a human

immune globulin, IgA less than 1 μg/mL in a 5% solution, for intravenous administration. In a particular embodiment, the active pharmaceutical ingredient comprises a human immune globulin composition.

GAMMAGARD LIQUID is a ready-for-use sterile, liquid preparation of highly purified and concentrated immunoglobulin G (IgG) antibodies. The distribution of the IgG subclasses is similar to that of normal plasma. The Fc and Fab functions are maintained in GAMMAGARD LIQUID. Pre-kallikrein activator activity is not detectable. GAMMAGARD LIQUID contains 100 milligram/mL protein. At least 98% of the protein is immune globulin, the average immunoglobulin A (IgA) concentration is 37 μg/mL, and immunoglobulin M is present in trace amounts. GAMMAGARD LIQUID contains a broad spectrum of IgG antibodies against bacterial and viral agents. Glycine (0.25M) serves as a stabilizing and buffering agent, and there are no added sugars, sodium or preservatives. The pH is 4.6 to 5.1. The osmolality is 240 to 300 mOsmol/kg, which is similar to physiological osmolality (285 to 295 mOsmol/kg).

GAMMAGARD LIQUID and GAMMAGARD S/D are manufactured from large pools of human plasma. IgG preparations are purified from plasma pools using a modified Cohn-Oncley cold ethanol fractionation process, as well as cation and anion exchange chromatography.

GAMMAGARD LIQUID and GAMMAGARD S/D supply a broad spectrum of opsonizing and neutralizing IgG antibodies against a wide variety of bacterial and viral agents. GAMMAGARD LIQUID and GAMMAGARD S/D also contain a spectrum of antibodies capable of interacting with and altering the activity of cells of the immune system as well as antibodies capable of reacting with cells such as erythrocytes.

In various embodiments, the pharmaceutical composition comprises human antihemophilic factor, factor VIII, preferably recombinant human antihemophilic factor factor VIII. In a particular embodiment, the pharmaceutical composition comprises ADVATE (Antihemophilic Factor (Recombinant) (rAHF)). rAHF is an antihemophilic factor (recombinant) for use in control and prevention of bleeding in adults and children with hemophilia A. The dosage and duration of treatment depend on the severity of factor VIII deficiency, the location and extent of the bleeding, and the patient's clinical condition. Careful control of replacement therapy is especially important in cases of major surgery or life-threatening bleeding episodes.

Recombinant antihemophilic factor is a purified glycoprotein consisting of 2,332 amino acids that is synthesized by a genetically engineered CHO cell line MCB 9710. In culture, the CHO cell line expresses recombinant antihemophilic factor (rAHF) into the cell culture medium. The rAHF is purified from the culture medium using a series of chromatography columns. The purification process includes an immunoaffinity chromatography step in which a monoclonal antibody directed against Factor VIII is employed to selectively isolate the rAHF from the medium. The cell culture and purification processes used in the manufacture of recombinant antihemophilic factor employ no additives of human or animal origin. The production process includes a dedicated, viral inactivation solvent-detergent treatment step. The recombinant antihemophilic factor synthesized by the CHO cells has the same biological effects on clotting as human Antihemophilic Factor (hAHF). Structurally the recombinant protein has a similar combination of heterogeneous heavy and light chains as found in AHF (Human). Amino acid analysis of the purified glycosylated protein demonstrated that it constitutes 2332 amino acids with a molecular mass of approximately 280 kDa.

ADVATE with 5 mL of Sterile Water for Injection, USP is available as a lyophilized powder in single-use vials containing nominally 250, 500, 1000, 1500, 2000, 3000 or 4000 IU. ADVATE with 2 mL of Sterile Water for Injection, USP is available as a lyophilized powder in single-use glass vials containing nominally 250, 500, 1000 or 1500 IU. Each vial of ADVATE is labeled with the rAHF activity expressed in International Units per vial. Biological potency is determined by an in vitro assay, which employs a factor VIII concentrate standard that is referenced to a WHO

International Standard for factor VIII concentrates. One International Unit, as defined by the WHO standard for blood coagulation factor VIII, human, is approximately equal to the level of factor VIII activity found in 1 mL of fresh pooled human plasma. The specific activity of ADVATE is 4000 to 10000 International Units per milligram of protein. The number of units of factor VIII administered is expressed in International Units (IU), which are related to the current WHO standard for factor VIII products.

Although ADVATE is packaged in a dry form in a glass vial, ADVATE is packaged with instructions to use a plastic syringe with the product because the proteins in the product tend to stick to the surface of glass syringes. When reconstituted with the provided sterile water for injection, the product contains the following stabilizers and excipients in the following amounts:

Approximate Concentration of Stabilizer and Excipient after Reconstitution

Stabilizer and Excipient	5 mL Reconstitution (for 250, 500, 1000, 1500, 2000, 3000, 4000 IU) Target	2 mL Reconstitution (for 250, 500, 1000, 1500 IU) Target
Tris (hydroxymethyl) aminomethane	10 mM	25 mM
Calcium Chloride	1.7 mM	4.2 mM
Mannitol	3.2% (w/v)	8% (w/v)
Sodium Chloride	90 mM	225 mM
α,α-Trehalose	0.8% (w/v)	2% (w/v)
Histidine	10 mM	25 mM
Glutathione (reduced)	0.08 mg/ml	0.2 mg/ml
Polysorbate 80	0.01% (w/v)	0.025% (w/v)

ADVATE (recombinant antihemophilic factor) is patented under U.S. Pat. Nos. 5,733,873; 5,854,021; 5,919,766; 5,955,448; 6,313,102; 6,586,573; 6,649,386; 7,087,723; and 7,247,707; and made according to the method of U.S. Pat. Nos. 5,470,954; 6,100,061; 6,475,725; 6,555,391; 6,936,441; 7,094,574; 7,253,262; and 7,381,796 (each of which is incorporated herein by reference in its entirety).

Coagulation diseases, such as von Willebrand Disease (VWD) and hemophilia, generally result from a deficiency in the coagulation cascade. "von Willebrand Disease" refers to the group of diseases caused by a deficiency of von Willebrand factor (VWF). Von Willebrand factor stimulates blood platelets aggregation and promotes platelet adhesion to the blood vessel wall, which is necessary for normal blood clotting. Von Willebrand factor also binds to and can increase the stability of factor VIII in circulation, thereby increasing the persistence of endogenous or administered factor VIII in circulation. Von Willebrand factor can be used to treat VWF deficiencies and factor VIII deficiencies (hemophilia A) by binding to and increasing the stability of factor VIII in circulation.

In various embodiments, the pharmaceutical composition comprises recombinant von Willebrand factor (rVWF). In specific embodiments, the pharmaceutical composition comprises BAX 111 (vonicog alfa also known as recombinant human von Willebrand Factor, or [618-threonine,709-aspartic acid]von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant) (CAS registry number 109319-16-6)). Vonicog alfa is currently undergoing clinical trials for the treatment of bleeding episodes in von Willebrand disease (see, e.g., ClinicalTrials.gov Identifier: NCT01410227 in the version available on the date of filing this application). The amino acid sequence of vonicog alfa is (SEQ ID NO: 1)

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SLSCRPPMVK LVCPADNLRA EGLECTKTCQ        50
NYDLECMSMG CVSGCLCPPG

MVRHENRCVA LERCPCFHQG KEYAPGETVK       100
IGCNTCVCRD RKWNCTDHVC

DATCSTIGMA HYLTFDGLKY LEPGECQYVL       150
VQDYCGSNPG TFRILVGNKG

CSHPSVKCKK RVTILVEGGE IELFDGEVNV       200
KRPMKDETHE EVVESGRYII

LLLGKALSVV WDRHLSISVV LKQTYQEKVC       250
GLCGNFDGIQ NNDLTSSNLQ

VEEDPVDFGN SWKVSSQCAD TRKVPLDSSP       300
ATCHNNIMKQ TMVDSSCRIL

TSDVFQDCNK LVDPEPYLDV CIYDTCSCES       350
IGDCACFCDT IAAYAHVCAQ

HGKVVTWRTA TLCPQSCEER NLRENGYECE       400
WRYNSCAPAC QVTCQHPEPL

ACPVQCVEGC HAHCPPGKIL DELLQTCVDP       450
EDCPVCEVAG RRFASGKKVT

LNPSDPEHCQ ICHCDVVNLT CEACQEPGGL       500
VVPPTDAPVS PTTLYVEDIS

EPPLHDFYCS RLLDLVFLLD GSSRLSEAEF       550
EVLKAFVVDM MERLRISQKW

VRVAVVEYHD GSHAYIGLKD RKRPSELRRI       600
ASQVKYAGSQ VASTSEVLKY

TLFQIFSKID RPEASRITLL LMASQEPQRM       650
SRNFVRYVQG LKKKKVIVIP

VGIGPHANLK QIRLTEKQAP ENKAFVLSSV       700
DELEQQRDEI VSYLCDLAPE

APPPTLPPDM AQVTVGPGLL GVSTLGPKRN       750
SMVLDVAFVL EGSDKIGEAD

FNRSKEFNEE VIQRMDVGQD SIHVTVLQYS       800
YMVTVEYPFS EAQSKGDILQ

RVREIRYQGG NRTNTGLALR YLSDHSFLVS       850
QGDREQAPNL VYMVTGNPAS

DEIKRLPGDI QVVPIGVGPN ANVQELERIG       900
WPNAPILIQD FETLPREAPD

LVLQRCCSGE GLQIPTLSPA PDCSQPLDVI       950
LLLDGSSSFP ASYFDEMKSF

AKAFISKANI GPRLTQVSVL QYGSITTIDV      1000
PWNVVPEKAH LLSLVDVMQR

EGGPSQIGDA LGFAVRYLTS EMHGARPGAS      1050
KAVVILVTDV SVDSVDAAAD
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AARSNRVTVF PIGIGDRYDA AQLRILAGPA      1100
GDSNVVKLQR IEDLPTMVTL

GNSFLHKLCS GEVRICMDED GNEKRPGDVW      1150
TLPDQCHTVT CQPDGQTLLK

SHRVNCDRGL RPSCPNSQSP VKVEETCGCR      1200
WTCPCVCTGS STRHIVTFDG

QNFKLTGSCS YVLFQNKEQD LEVILHNGAC      1250
SPGARQGCMK SIEVKHSALS

VELHSDMEVT VNGRLVSVPY VGGNMEVNVY      1300
GAIMHEVRFN HLGHIFTFTP

QNNEEQLQLS PKTFASKTYG LCGICDENGA      1350
NDFMLRDGTV TTDWKTLVQE

WTVQRPGQTC QPILEEQCLV PDSSHCQVLL      1400
LPLFAECHKV LAPATFYAIC

QQDSCHQEQV CEVIASYAHL CRTNGVCVDW      1450
RTPDFCAMSC PPSLVYNHCE

HGCPRHCDGN VSSCGDHPSE GCFCPPDKVM      1500
LEGSCVPEEA CTQCIGEDGV

QHQFLEAWVP DMQPCQICTC LSGRKVNCTT      1550
QPCPTAKAPT CGLCEVARLR

QNADQCCPEY ECVCDPVSCD LPPVPHCERG      1600
LQPTLTNPGE CRPNRTCACR

KEECKRVSPP SCPPHRLPTL RKTQCCDEYE      1650
CACNCVNSTV SCPLGYLAST

ATNDCGCTTT TCLPDKVCVH RSTIYPVGQF      1700
WEEGCDVCTC TDMEDAVMGL

RVAQCSQKPC EDSCRSGFTY VLHEGECCGR      1750
CLPSACEVVT GSPRGDSQSS

WKSVGSQWAS PENPCLINEC VRVKEEVFIQ      1800
QRNVSCPQLE VPVCPSGFQL

SCKTSACCPS CRCERMEACM LNGTVIGPGK      1850
TVMIDVCTTC RCMVQVGVIS

GFKLECRKTT CNPCPLGYKE ENNTGECCGR      1900
CLPTACTIQL RGGQIMTLKR

DETLQDGCDT HFCKVNERGE YFWEKRVTGC      1950
PPFDEHKCLA EGGKIMKIPG

TCCDTCEEPE CNDITARLQY VKVGSCKSEV      2000
EVDIHYCQGK CASKAMYSID

INDVQDQCSC CSPTRTEPMQ VALHCTNGSV      2050
VYHEVLNAME CKCSPRKCSK
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In various embodiments, the pharmaceutical composition comprises an adeno-associated viral vector, for example a self-complementary adeno-associated viral vector. In an embodiment, the adeno-associated viral vector is for expression of a human clotting factor, e.g., for expression of one or more of human factor VIII, human factor IX, human factor X, or human von Willebrand factor. In one embodiment, the pharmaceutical composition is a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette, sscAAV2/8-LP1-hFIXco, for the treatment of hemophilia B. Transduction with recombinant adenoassociated virus (AAV) vectors is limited by the need to convert its single-stranded (ss) genome to transcriptionally active double-stranded (ds) forms. For AAV mediated hemophilia B (HB) gene therapy, this obstacle has been overcome by constructing a liver-restricted mini-human factor IX (hFIX) expression cassette that can be packaged as complementary dimers within individual AAV particles.

sscAAV2/8-LP1-hFIXco is currently undergoing clinical trials for the treatment of bleeding episodes in hemophilia B (see, e.g., ClinicalTrials.gov Identifier: NCT00979238, incorporated herein by reference in the version available on the date of filing of this application, and Nathwani et al., 2011, Adenovirus-Associated Virus Vector-Mediated Gene Transfer in Hemophilia B. *NEJM.* 365:2357-2365, incorporated herein by reference). Generation of the AAV vector is described in Nathwani et al., 2006 (Self-complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. *Blood* 107:2653-2661, incorporated herein by reference). The nucleic acid sequence (SEQ ID NO:2) and the amino acid sequence (SEQ ID NO:3) encoded by sscAAV2/8-LP1-hFIXco are:

Hemophilia B is an X-linked bleeding disorder that results from a defect in the gene encoding coagulation factor IX (FIX), a serine protease that is critical for blood clotting. Persons with severe hemophilia B have functional FIX levels that are less than 1% of normal values and have frequent bleeding episodes, which are associated with crippling arthropathy and early death. Current treatment involves frequent intravenous injections of FIX protein concentrate (i.e., two to three times a week). However, this treatment is prophylactic rather than curative, is extremely expensive, and is associated with inhibitor formation. Somatic gene therapy for hemophilia B offers the potential for a cure through continuous endogenous production of FIX after a single administration of vector, especially since a small rise in circulating FIX to at least 1% of normal levels can substantially ameliorate the bleeding phenotype.

As described in Nathwani et al., 2011, six patients with severe hemophilia B (factor IX activity, <1% of normal values) were infused a single dose of the scAAV2/8-LP1-hFIXco vector expressing a codon-optimized human factor IX transgene in a peripheral vein. AAV-mediated expression of factor IX at 2 to 11% of normal levels was observed in all participants. Four of the six discontinued factor IX prophylaxis and remained free of spontaneous hemorrhage; in the other two, the interval between prophylactic injections was increased. The study demonstrated that peripheral-vein infusion of scAAV2/8-LP1-hFIXco resulted in factor IX transgene expression at levels sufficient to improve the bleeding phenotype, with few side effects.

Degradation and Stability of Pharmaceutical Compositions

According to the present invention, delamination resistant pharmaceutical containers comprising a glass composition provide for improved resistance to degradation of, improved stability of, improved resistance to inactivation of, and improved maintenance of levels of a pharmaceutical composition having at least one active pharmaceutical ingredient, for example, GAMMAGARD LIQUID or GAMMAGARD S/D (a human immune globulin); ADVATE (human Antihemophilic Factor (Recombinant), (rAHF)); BAX 111 (vonicog alfa, also known as recombinant human von Willebrand factor (rVWF), or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T, 1472H>D variant)); or an adeno-associated viral vector containing a liver-specific human factor IX expression cassette, e.g., sscAAV2/8-LP1-hFIXco.

In one embodiment of the present invention, the delamination resistant pharmaceutical containers provide improved stability to pharmaceutical compositions contained therein, for example, GAMMAGARD LIQUID or GAMMAGARD S/D (a human immune globulin); ADVATE (human Antihemophilic Factor (Recombinant), (rAHF)); BAX 111 (vonicog alfa, also known as recombinant human von Willebrand factor (rVWF), or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or an adeno-associated viral vector containing a liver-specific human factor IX expression cassette, e.g., sscAAV2/8-LP1-hFIXco. As used herein, the term "stability" refers to the ability of an active pharmaceutical ingredient to essentially retain its physical, chemical and conformational identity and integrity upon storage in the pharmaceutical containers of the invention. Stability is associated with the ability of an active pharmaceutical ingredient to retain its potency and efficacy over a period of time. Instability of an active pharmaceutical ingredient may be associated with, for example, chemical or physical degradation, fragmentation, conformational change, increased toxicity, aggregation (e.g., to form higher order polymers), deglycosylation, modification of glycosylation, oxidation, hydrolysis, or any other structural, chemical or physical modification. Such physical, chemical and/or conformational changes often result in reduced activity or inactivation of the active pharmaceutical ingredient, for example, such that at least one biological activity of the active pharmaceutical ingredient is reduced or eliminated. Alternatively or in addition, such physical, chemical and/or conformational changes often result in the formation of structures toxic to the subject to whom the pharmaceutical composition is administered.

The pharmaceutical containers of the present invention maintain stability of the pharmaceutical compositions, in part, by minimizing or eliminating delamination of the glass composition which forms, at least in part, the pharmaceutical container. In addition, the pharmaceutical containers of the present invention maintain stability of the pharmaceutical compositions, in part, by reducing or preventing the interaction of the active pharmaceutical ingredient with the pharmaceutical container and/or delaminated particles resulting therefrom. By minimizing or eliminating delamination and, further, by reducing or preventing interaction, the pharmaceutical containers thereby reduce or prevent the destabilization of the active pharmaceutical ingredient as found in, for example, GAMMAGARD LIQUID or GAMMAGARD S/D (human immune globulin); ADVATE (human Antihemophilic Factor (Recombinant), (rAHF)); BAX 111 (vonicog alfa, recombinant human von Willebrand Factor (rVWF), or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or an adeno-associated viral vector containing a liver-specific human factor IX expression cassette, e.g., sscAAV2/8-LP1-hFIXco.

The pharmaceutical containers of the present invention provide the additional advantage of preventing loss of active pharmaceutical ingredients. For example, by reducing or preventing the interaction of and, thus, the adherence of, the active pharmaceutical ingredient with the pharmaceutical container and/or delaminated particles resulting therefrom, the level of active pharmaceutical ingredient available for administration to a subject is maintained, as found in, for example, GAMMAGARD LIQUID or GAMMAGARD S/D (human immune globulin); ADVATE (human Antihemophilic Factor (Recombinant), (rAHF)); BAX 111 (vonicog alfa, recombinant human von Willebrand Factor (RVWF), or [618-threonine,709-aspartic acid] von Willebrand factor *Homo sapiens* (1381A>T,1472H>D variant)); or an adeno-associated viral vector containing a liver-specific human factor IX expression cassette, e.g., sscAAV2/8-LP1-hFIXco.

In one embodiment of the present invention, the pharmaceutical composition has a high pH. According to the present invention, it has been discovered that high pHs serve to increase delamination of glass compositions. Accordingly, the pharmaceutical containers of the present invention are particularly suitable for storing and maintaining pharmaceutical compositions having a high pH, for example, pharmaceutical compositions having a pH between about 7 and about 11, between about 7 and about 10, between about 7 and about 9, or between about 7 and about 8.

In additional embodiments, the pharmaceutical containers of the present invention are particularly suitable for storing and maintaining pharmaceutical compositions having phosphate or citrate based buffers. According to the present invention, it has been discovered that phosphate or citrate based buffers serve to increase delamination of glass compositions. According in particular embodiments, the pharmaceutical composition includes a buffer comprising a salt of citrate, e.g., sodium citrate, or SSC. In other embodiments, the pharmaceutical composition includes a buffer comprising a salt of phosphate, e.g., mono or disodium phosphate.

In additional embodiments, the pharmaceutical containers of the present invention are particularly suitable for storing and maintaining active pharmaceutical ingredient that needs to be subsequently formulated. In other embodiments, the pharmaceutical containers of the present invention are particularly suitable for storing and maintaining a lyophilized pharmaceutical composition or active pharmaceutical ingredient that requires reconstitution, for example, by addition of saline.

Assaying for Delamination of Pharmaceutical Containers

As noted above, delamination may result in the release of silica-rich glass flakes into a solution contained within the glass container after extended exposure to the solution. Accordingly, the resistance to delamination may be characterized by the number of glass particulates present in a solution contained within the glass container after exposure to the solution under specific conditions. In order to assess the long-term resistance of the glass container to delamination, an accelerated delamination test was utilized. The test consisted of washing the glass container at room temperature for 1 minute and depyrogenating the container at about 320° C. for 1 hour. Thereafter a solution of 20 mM glycine with a pH of 10 in water is placed in the glass container to 80-90% fill, the glass container is closed, and rapidly heated to 100° C. and then heated from 100° C. to 121° C. at a ramp rate of 1 deg/min at a pressure of 2 atmospheres. The glass container and solution are held at this temperature for 60 minutes, cooled to room temperature at a rate of 0.5 deg./min and the heating cycle and hold are repeated. The glass container is then heated to 50° C. and held for two days for elevated temperature conditioning. After heating, the glass container is dropped from a distance of at least 18" onto a firm surface, such as a laminated tile floor, to dislodge any flakes or particles that are weakly adhered to the inner surface of the glass container.

Thereafter, the solution contained in the glass container is analyzed to determine the number of glass particles present per liter of solution. Specifically, the solution from the glass container is directly poured onto the center of a Millipore Isopore Membrane filter (Millipore #ATTP02500 held in an assembly with parts #AP1002500 and #M000025A0) attached to vacuum suction to draw the solution through the filter within 10-15 seconds. Particulate flakes are then counted by differential interference contrast microscopy (DIC) in the reflection mode as described in "Differential interference contrast (DIC) microscopy and modulation contrast microscopy" from Fundamentals of light microscopy and digital imaging. New York: Wiley-Liss, pp 153-168. The field of view is set to approximately 1.5 mm×1.5 mm and particles larger than 50 microns are counted manually. There are 9 such measurements made in the center of each filter membrane in a 3×3 pattern with no overlap between images. A minimum of 100 mL of solution is tested. As such, the solution from a plurality of small containers may be pooled to bring the total amount of solution to 100 mL. If the containers contain more than 10 mL of solution, the entire amount of solution from the container is examined for the presence of particles. For containers having a volume greater than 10 mL, the test is repeated for a trial of 10 containers formed from the same glass composition under the same processing conditions and the result of the particle count is averaged for the 10 containers to determine an average particle count. Alternatively, in the case of small containers, the test is repeated for a trial of 10 sets of 10 mL of solution, each of which is analyzed and the particle count averaged over the 10 sets to determine an average particle count. Averaging the particle count over multiple containers accounts for potential variations in the delamination behavior of individual containers. Table 7 summarizes some non-limiting examples of sample volumes and numbers of containers for testing is shown below:

TABLE 7

Table of Exemplary Test Specimens

Nominal Vial Capacity (mL)	Vial Max Volume (mL)	Minimum Solution per Vial (mL)	Number of Vials in a Trial	Number of Trials	Total solution Tested (mL)
2	4	3.2	4	10	128
3.5	7	5.6	2	10	112
4	6	4.8	3	10	144
5	10	8	2	10	160
6	10	8	2	10	160
8	11.5	9.2	2	10	184
10	13.5	10.8	1	10	108
20	26	20.8	1	10	208
30	37.5	30	1	10	300
50	63	50.4	1	10	504

It should be understood that the aforementioned test is used to identify particles which are shed from the interior wall(s) of the glass container due to delamination and not tramp particles present in the container from forming processes or particles which precipitate from the solution enclosed in the glass container as a result of reactions between the solution and the glass. Specifically, delamination particles may be differentiated from tramp glass particles based on the aspect ratio of the particle (i.e., the ratio of the width of the particle to the thickness of the particle). Delamination produces particulate flakes or lamellae which are irregularly shaped and are typically >50 μm in diameter but often >200 μm. The thickness of the flakes is usually greater than about 100 nm and may be as large as about 1 μm. Thus, the minimum aspect ratio of the flakes is typically >50. The aspect ratio may be greater than 100 and sometimes greater than 1000. Particles resulting from delamination processes generally have an aspect ratio which is generally greater than about 50. In contrast, tramp glass particles will generally have a low aspect ratio which is less than about 3. Accordingly, particles resulting from delamination may be differentiated from tramp particles based on aspect ratio during observation with the microscope. Vali-

dation results can be accomplished by evaluating the heel region of the tested containers. Upon observation, evidence of skin corrosion/pitting/flake removal, as described in "Nondestructive Detection of Glass Vial Inner Surface Morphology with Differential Interference Contrast Microscopy" from Journal of Pharmaceutical Sciences 101(4), 2012, pages 1378-1384, is noted.

In the embodiments described herein, glass containers which average less than 3 glass particles with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered "delamination resistant." In the embodiments described herein, glass containers which average less than 2 glass particles with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered "delamination-stable." In the embodiments described herein, glass containers which average less than 1 glass particle with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered "delamination-proof." In the embodiments described herein, glass containers which have 0 glass particles with a minimum width of 50 μm and an aspect ratio of greater than 50 per trial following accelerated delamination testing are considered "delamination-free".

Assessing Stability of Pharmaceutical Compositions

As set forth above, any of a variety of active pharmaceutical ingredients can be incorporated within the claimed pharmaceutical container including, for example, a small molecule, a polypeptide mimetic, a biologic, an antisense RNA, a small interfering RNA (siRNA), etc. These active ingredients degrade in varying manners and, thus, assessing the stability thereof in the pharmaceutical containers of the present invention requires different techniques.

Depending on the nature of the active pharmaceutical ingredient, the stability, maintenance and/or continued efficacy of the pharmaceutical compositions contained within the delamination resistant pharmaceutical containers of the present invention can be evaluated as follows.

Biologics API are often susceptible to degradation and/or inactivation arising from various factors, including pH, temperature, temperature cycling, light, humidity, etc. Biologics API are further susceptible to degradation, inactivation or loss, arising from interaction of the pharmaceutical composition with the pharmaceutical container, or delaminants leeching therefrom. For example, biologics may undergo physical degradation which may render the resulting pharmaceutical composition inactive, toxic or insufficient to achieve the desired effect. Alternatively, or in addition, biologics may undergo structural or conformational changes that can alter the activity of the API, with or without degradation. For example, proteins may undergo unfolding which can result in effective loss and inactivity of the API. Alternatively, or in addition, biologics may adhere to the surface of the container, thereby rendering the API administered to the subject insufficient to achieve the desired effect, e.g., therapeutic effect.

(i) General Methods for Investigation of Biologic Compound Degradation

Depending on the size and complexity of the biologic, methods for analysis of degradation of non-biologic, small molecule API may be applied to biologics. For example, peptides and nucleic acids can be analyzed using any of a number of chromatography and spectrometry techniques applicable to small molecules to determine the size of the molecules, either with or without protease or nuclease digestion. However, as proper secondary and tertiary structures are required for the activity of biologics, particularly protein biologics, confirmation of molecular weight is insufficient to confirm activity of biologics. Protein biologics containing complex post-translational modifications, e.g., glycosylation, are less amenable to analysis using chromatography and spectrometry. Moreover, complex biologics, e.g., vaccines which can include complex peptide mixtures, attenuated or killed viruses, or killed cells, are not amenable to analysis by most chromatography or spectrometry methods.

(ii) In Vitro Functional Assays for Investigation of Compound Stability

One or more in vitro assays, optionally in combination with one or more in vivo assays, can be used to assess the stability and activity of the API. Functional assays to determine API stability can be selected based on the structural class of the API and the function of the API. Exemplary assays are provided below to confirm the activity of the API after stability and/or stress testing. It is understood that assays should be performed with the appropriate controls (e.g., vehicle controls, control API not subject to stress or stability testing) with a sufficient number of dilutions and replicate samples to provide data with sufficient statistical significance to detect changes in activity of 10% or less, preferably 5% or less, 4% or less, more preferably 3% or less, 2% or less, or 1% or less, as desired. Such considerations in the art are well understood.

For example, antibody based therapeutics, regardless of the disease or condition to be treated, can be assayed for stability and activity using assays that require specific binding of the antibody to its cognate antigen, e.g., ELISA. The antigen used in the ELISA should have the appropriate conformational structure as would be found in vivo. Antibody based API are used, for example, for the treatment of cancer and inflammatory diseases including autoimmune diseases.

ELISA assays to assay the concentration of a protein biologic API are commercially available from a number of sources, e.g., R&D Systems, BD Biosciences, AbCam, Pierce, Invitrogen.

API are frequently targeted to receptors, particularly cell surface receptors. Receptor binding assays can be used to assess the activity of such agents. API that bind cell surface receptors can be agonists, antagonists or allosteric modulators. API that bind cell surface receptors need not bind the same location as the native ligand to modulate, for example, inhibit or enhance, signaling through the receptor. Depending on the activity of the API, an appropriate assay can be selected, e.g., assay for stimulation of receptor signaling when the API is a receptor agonist; and inhibition assay in which binding of an agonist, e.g., inhibition of activation by a receptor agonist by the API. Such assays can be used regardless of the disease(s) or condition(s) to be treated with the API. Modulation of cellular activity, e.g., cell proliferation, apoptosis, cell migration, modulation of expression of genes or proteins, differentiation, tube formation, etc. is assayed using routine methods. In other assay methods, a reporter construct is used to indicate activation of the receptor. Such methods are routine in the art. APIs that bind to cell surface receptors are used, for example, as anti-cancer agents, anti-diabetic agents, anti-inflammatory agents for the treatment of inflammatory mediated diseases including autoimmune disorders, anti-angiogenic agents, anti-cholinergic agents, bone calcium regulators, muscle and vascular tension regulators, and psychoactive agents.

Modulators of cell proliferation can be assayed for activity using a cell proliferation assays. For example, cell

proliferation is induced using anti-anemic agents or stimulators of hematopoietic cell growth. Anti-proliferative agents, e.g., cytotoxic agents, anti-neoplastic agents, chemotherapeutic agents, cytostatic agents, antibiotic agents, are used to inhibit growth of various cell types. Some anti-inflammatory agents also act by inhibiting proliferation of immune cells, e.g., blast cells. In proliferation assays, replicate wells containing the same number of cells are cultured in the presence of the API. The effect of the API is assessed using, for example, microscopy or fluorescence activated cell sorting (FACS) to determine if the number of cells in the sample increased or decreased in response to the presence of the API. It is understood that the cell type selected for the proliferation assay is dependent on the specific API to be tested.

Modulators of angiogenesis can be assayed using cell migration and/or tube formation assays. For cell migration assays, human vascular endothelial cells (HUVECs) are cultured in the presence of the API in transwell devices. Migration of cells through the device at the desired time intervals is assessed. Alternatively, 3-dimensional HUVECs cultures in MATRIGEL can be assessed for tube formation. Anti-angiogenic agents are used, for example, for the treatment of cancer, macular degeneration, and diabetic retinopathy.

Anti-inflammatory API can be assayed for their effects on immune cell stimulation as determined, for example, by modulation of one or more of cytokine expression and secretion, antigen presentation, migration in response to cytokine or chemokine stimulation, and immune cell proliferation. In such assays, immune cells are cultured in the presence of the API and changes in immune cell activity are determined using routine methods in the art, e.g., ELISA and cell imaging and counting.

Anti-diabetic API can be assayed for their effects on insulin signaling, including insulin signaling in response to modulated glucose levels, and insulin secretion. Insulin signaling can be assessed by assessing kinase activation in response to exposure to insulin and/or modulation of glucose levels. Insulin secretion can be assessed by ELISA assay.

Modulators of blood clotting, i.e., fibrinolytics, anti-fibrinolytics, and anti-coagulants, can be assayed for their effects using an INR assay on serum by measuring prothrombin time to determine a prothrombin ratio. Time to formation of a clot is assayed in the presence or absence of the API.

Modulators of muscle or vascular tone can be assayed for their effects using vascular or muscle explants. The tissue can be placed in a caliper for detection of changes in length and/or tension. Whole coronary explants can be used to assess the activity of API on heart. The tissue is contacted with the API, and optionally agents to alter vascular tone (e.g., K^+, Ca^{++}). The effects of the API on length and/or tension of the vasculature or muscle is assessed.

Psychoactive agents can act by modulation of neurotransmitter release and/or recycling. Neuronal cells can be incubated in the presence of an API and stimulated to release neurotransmitters. Neurotransmitter levels can be assessed in the culture medium collected at defined time points to detect alterations in the level of neurotransmitter present in the media. Neurotransmitters can be detected, for example, using ELISA, LC/MS/MS, or by preloading the vesicles with radioactive neurotransmitters to facilitate detection.

(iii) In Vivo Assays for Investigation of Compound Stability

In addition to in vitro testing for compound stability, API can also be tested in vivo to confirm the stability of the API after storage and/or stress testing. For example, some API are not amenable to testing using in vitro assays due to the complexity of the disease state or the complexity of the response required. For example, psychoactive agents, e.g., antipsychotic agents, anti-depressant agents, nootropic agents, immunosuppressant agents, vasotherapeutic agents, muscular dystrophy agents, central nervous system modulating agents, antispasmodic agents, bone calcium regenerating agents, anti-rheumatic agents, anti-hyperlipidemic agents, hematopoietic proliferation agents, growth factors, vaccine agents, and imaging agents, may not be amenable to full functional characterization using in vitro models. Moreover, for some agents, factors that may not alter in vitro activity may alter activity in vivo, e.g., antibody variable domains may be sufficient to block signaling through a receptor, but the Fc domains may be required for efficacy in the treatment of disease. Further, changes in stability may result in changes in pharmacokinetic properties of an API (e.g., half-life, serum protein binding, tissue distribution, CNS permeability). Finally, changes in stability may result in the generation of toxic degradation or reaction products that would not be detected in vivo. Therefore, confirmation of pharmacokinetic and pharmacodynamic properties and toxicity in vivo is useful in conjunction with stability and stress testing.

(iv) Pharmacokinetic Assays

Pharmacokinetics includes the study of the mechanisms of absorption and distribution of an administered drug, the rate at which a drug action begins and the duration of the effect, the chemical changes of the substance in the body (e.g. by metabolic enzymes such as CYP or UGT enzymes) and the effects and routes of excretion of the metabolites of the drug. Pharmacokinetics is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as the ADME scheme:

Absorption—the process of a substance entering the blood circulation.

Distribution—the dispersion or dissemination of substances throughout the fluids and tissues of the body.

Metabolism (or Biotransformation)—the irreversible transformation of parent compounds into daughter metabolites.

Excretion—the removal of the substances from the body. In rare cases, some drugs irreversibly accumulate in body tissue.

Elimination is the result of metabolism and excretion.

Pharmacokinetics describes how the body affects a specific drug after administration. Pharmacokinetic properties of drugs may be affected by elements such as the site of administration and the dose of administered drug, which may affect the absorption rate. Such factors cannot be fully assessed using in vitro models.

The specific pharmacokinetic properties to be assessed for a specific API in stability testing will depend, for example, on the specific API to be tested. In vitro pharmacokinetic assays can include assays of drug metabolism by isolated enzymes or by cells in culture. However, pharmacokinetic analysis typically requires analysis in vivo.

As pharmacokinetics are not concerned with the activity of the drug, but instead with the absorption, distribution, metabolism, and excretion of the drug, assays can be performed in normal subjects, rather than subjects suffering from a disease or condition for which the API is typically administered, by administration of a single dose of the API to the subject. However, if the subject to be treated with the API has a condition that would alter the metabolism or excretion of the API, e.g., liver disease, kidney disease,

testing of the API in an appropriate disease model may be useful. Depending on the half life of the compound, samples (e.g., serum, urine, stool) are collected at predetermined time points for at least two, preferably three half-lives of the API, and analyzed for the presence of the API and metabolic products of the API. At the end of the study, organs are harvested and analyzed for the presence of the API and metabolic products of the API. The pharmacokinetic properties of the API subjected to stability and/or stress testing are compared to API not subjected to stability or stress testing and other appropriate controls (e.g., vehicle control). Changes in pharmacokinetic properties as a result of stability and/or stress testing are determined.

(v) Pharmacodynamic Assays

Pharmacodynamics includes the study of the biochemical and physiological effects of drugs on the body or on microorganisms or parasites within or on the body and the mechanisms of drug action and the relationship between drug concentration and effect. Due to the complex nature of many disease states and the actions of many API, the API should be tested in vivo to confirm the desired activity of the agent. Mouse models for a large variety of disease states are known and commercially available (see, e.g., jaxmice.jax-.org/query/f?p=205:1:989373419139701::::P1_ADV:1). A number of induced models of disease are also known. Agents can be tested on the appropriate animal model to demonstrate stability and efficacy of the API on modulating the disease state.

(vi) Specific Immune Response Assay

Vaccines produce complex immune responses that are best assessed in vivo. The specific potency assay for a vaccine depends, at least in part, on the specific vaccine type. The most accurate predictions are based on mathematical modeling of biologically relevant stability-indicating parameters. For complex vaccines, e.g., whole cell vaccines, whole virus vaccines, complex mixtures of antigens, characterization of each component biochemically may be difficult, if not impossible. For example, when using a live, attenuated virus vaccine, the number of plaque forming units (e.g., mumps, measles, rubella, smallpox) or colony forming units (e.g., *S. typhi*, TY21a) are determined to confirm potency after storage. Chemical and physical characterization (e.g., polysaccharide and polysaccharide-protein conjugate vaccines) is performed to confirm the stability and activity of the vaccine. Serological response in animals to inactivated toxins and/or animal protection against challenge (e.g., rabies, anthrax, diphtheria, tetanus) is performed to confirm activity of vaccines of any type, particularly when the activity of the antigen has been inactivated. In vivo testing of vaccines subjected to stability and/or stress testing is performed by administering the vaccine to a subject using the appropriate immunization protocol for the vaccine, and determining the immune response by detection of specific immune cells that respond to stimulation with the antigen or pathogen, detection of antibodies against the antigen or pathogen, or protection in an immune challenge. Such methods are well known in the art. Vaccines include, but are not limited to, meningococcal B vaccine, hepatitis A and B vaccines, human papillomavirus vaccine, influenza vaccine, herpes zoster vaccine, and pneumococcal vaccine.

(vii) Toxicity Assays

Degradation of API can result in in the formation of toxic agents. Toxicity assays include the administration of doses, typically far higher than would be used for therapeutic applications, to detect the presence of toxic products in the API. Toxicity assays can be performed in vitro and in vivo and are frequently single, high dose experiments. After administration of the compound, in addition to viability, organs are harvested and analyzed for any indication of toxicity, especially organs involved with clearance of API, e.g., liver, kidneys, and those for which damage could be catastrophic, e.g., heart, brain. The toxicologic properties of the API subjected to stability and/or stress testing are compared to API not subjected to stability or stress testing and other appropriate controls (e.g., vehicle control). Changes in toxicologic properties as a result of stability and/or stress testing are determined

In accordance with present invention, the degradation, alteration or depletion of API contained within a delamination resistant pharmaceutical container of the present invention can be assessed by a variety of physical techniques. Indeed, in various aspects of the invention, the stability and degradation caused by the interaction of API with the container or delaminants thereof, or changes in concentration or amount of the API in a container can be assessed using techniques as follows. Such methods include, e.g., X-Ray Diffraction (XRPD), Thermal Analysis (such as Differential Scanning calorimetry (DSC), Thermogravimetry (TG) and Hot-Stage Microscopy (HSM), chromatography methods (such as High-Performance Liquid Chromatography (HPLC), Column Chromatography (CC), Gas Chromatography (GC), Thin-Layer Chromatography (TLC), and Super Critical Phase Chromatograph (SFC)), Mass Spectroscopy (MS), Capillary Electrophoresis (CE), Atomic Spectroscopy (AS), vibrational spectroscopy (such as Infrared Spectroscopy (IR)), Luminescence Spectroscopy (LS), and Nuclear Magnetic Resonance Spectroscopy (NMR).

In the case of pharmaceutical formulations where the API is not in solution or needs to be reconstituted into a different medium, XRPD may be a method for analyzing degradation. In ideal cases, every possible crystalline orientation is represented equally in a non-liquid sample.

Powder diffraction data is usually presented as a diffractogram in which the diffracted intensity I is shown as function either of the scattering angle 2θ or as a function of the scattering vector q. The latter variable has the advantage that the diffractogram no longer depends on the value of the wavelength λ. Relative to other methods of analysis, powder diffraction allows for rapid, non-destructive analysis of multi-component mixtures without the need for extensive sample preparation. Deteriorations of an API may be analyzed using this method, e.g., by comparing the diffraction pattern of the API to a known standard of the API prior to packaging.

Thermal methods of analysis may include, e.g., differential scanning calorimetry (DSC), thermogravimetry (TG), and hot-stage microscopy (HSM). All three methods provide information upon heating the sample. Depending on the information required, heating can be static or dynamic in nature.

Differential scanning calorimetry monitors the energy required to maintain the sample and a reference at the same temperature as they are heated. A plot of heat flow (W/g or J/g) versus temperature is obtained. The area under a DSC peak is directly proportional to the heat absorbed or released and integration of the peak results in the heat of transition.

Thermogravimetry (TG) measures the weight change of a sample as a function of temperature. A total volatile content of the sample is obtained, but no information on the identity of the evolved gas is provided. The evolved gas must be identified by other methods, such as gas chromatography, Karl Fisher titration (specifically to measure water), TG-mass spectroscopy, or TG-infrared spectroscopy. The temperature of the volatilization and the presence of steps in the

TG curve can provide information on how tightly water or solvent is held in the lattice. If the temperature of the TG volatilization is similar to an endothermic peak in the DSC, the DSC peak is likely due or partially due to volatilization. It may be necessary to utilize multiple techniques to determine if more than one thermal event is responsible for a given DSC peak.

Hot-Stage Microscopy (HSM) is a technique that supplements DSC and TG. Events observed by DSC and/or TG can be readily characterized by HSM. Melting, gas evolution, and solid-solid transformations can be visualized, providing the most straightforward means of identifying thermal events. Thermal analysis can be used to determine the melting points, recrystallizations, solid-state transformations, decompositions, and volatile contents of pharmaceutical materials.

Other methods to analyze degradation or alteration of API and excipients are infrared (IR) and Raman spectroscopy. These techniques are sensitive to the structure, conformation, and environment of organic compounds. Infrared spectroscopy is based on the conversion of IR radiation into molecular vibrations. For a vibration to be IR-active, it must involve a changing molecular dipole (asymmetric mode). For example, vibration of a dipolar carbonyl group is detectable by IR spectroscopy. Whereas IR has been traditionally used as an aid in structure elucidation, vibrational changes also serve as probes of intermolecular interactions in solid materials.

Raman spectroscopy is based on the inelastic scattering of laser radiation with loss of vibrational energy by a sample. A vibrational mode is Raman active when there is a change in the polarizability during the vibration. Symmetric modes tend to be Raman-active. For example, vibrations about bonds between the same atom, such as in alkynes, can be observed by Raman spectroscopy.

NMR spectroscopy probes atomic environments based on the different resonance frequencies exhibited by nuclei in a strong magnetic field. Many different nuclei are observable by the NMR technique, but those of hydrogen and carbon atoms are most frequently studied. Solid-state NMR measurements are extremely useful for characterizing the crystal forms of pharmaceutical solids. Nuclei that are typically analyzed with this technique include those of 13C, 31P, 15N, 25Mg, and 23Na.

Chromatography is a general term applied to a wide variety of separation techniques based on the sample partitioning between a moving phase, which can be a gas, liquid, or supercritical fluid, and a stationary phase, which may be either a liquid or a solid. Generally, the crux of chromatography lies in the highly selective chemical interactions that occur in both the mobile and stationary phases. For example, depending on the API and the separation required, one or more of absorption, ion-exchange, size-exclusion, bonded phase, reverse, or normal phase stationary phases may be employed.

Mass spectrometry (MS) is an analytical technique that works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios. Based on this analysis method, one can determine, e.g., the isotopic composition of elements in an API and determine the structure of the API by observing its fragmentation pattern.

It would be understood that the foregoing methods do not represent a comprehensive list of means by which one can analyze possible deteriorations, alterations, or concentrations of certain APIs. Therefore, it would be understood that other methods for determining the physical amounts and/or characteristics of an API may be employed. Additional methods may include, but are not limited to, e.g., Capillary Electrophoresis (CE), Atomic Spectroscopy (AS), and Luminescence Spectroscopy (LS).

EXAMPLES

The embodiments of the delamination resistant pharmaceutical containers described herein will be further clarified by the following examples.

Example 1

Six exemplary inventive glass compositions (compositions A-F) were prepared. The specific compositions of each exemplary glass composition are reported below in Table 8. Multiple samples of each exemplary glass composition were produced. One set of samples of each composition was ion exchanged in a molten salt bath of 100% KNO_3 at a temperature of 450° C. for at least 5 hours to induce a compressive layer in the surface of the sample. The compressive layer had a surface compressive stress of at least 500 MPa and a depth of layer of at least 45 μm.

The chemical durability of each exemplary glass composition was then determined utilizing the DIN 12116 standard, the ISO 695 standard, and the ISO 720 standard described above. Specifically, non-ion exchanged test samples of each exemplary glass composition were subjected to testing according to one of the DIN 12116 standard, the ISO 695 standard, or the ISO 720 standard to determine the acid resistance, the base resistance or the hydrolytic resistance of the test sample, respectively. The hydrolytic resistance of the ion exchanged samples of each exemplary composition was determined according to the ISO 720 standard. The average results of all samples tested are reported below in Table 8.

As shown in Table 8, exemplary glass compositions A-F all demonstrated a glass mass loss of less than 5 mg/dm^2 and greater than 1 mg/dm^2 following testing according to the DIN 12116 standard with exemplary glass composition E having the lowest glass mass loss at 1.2 mg/dm^2. Accordingly, each of the exemplary glass compositions were classified in at least class S3 of the DIN 12116 standard, with exemplary glass composition E classified in class S2. Based on these test results, it is believed that the acid resistance of the glass samples improves with increased SiO_2 content.

Further, exemplary glass compositions A-F all demonstrated a glass mass loss of less than 80 mg/dm^2 following testing according to the ISO 695 standard with exemplary glass composition A having the lowest glass mass loss at 60 mg/dm^2. Accordingly, each of the exemplary glass compositions were classified in at least class A2 of the ISO 695 standard, with exemplary glass compositions A, B, D and F classified in class A1. In general, compositions with higher silica content exhibited lower base resistance and compositions with higher alkali/alkaline earth content exhibited greater base resistance.

Table 8 also shows that the non-ion exchanged test samples of exemplary glass compositions A-F all demonstrated a hydrolytic resistance of at least Type HGA2 following testing according to the ISO 720 standard with exemplary glass compositions C—F having a hydrolytic resistance of Type HGA1. The hydrolytic resistance of exemplary glass compositions C—F is believed to be due to higher amounts of SiO_2 and the lower amounts of Na_2O in the glass compositions relative to exemplary glass compositions A and B.

Moreover, the ion exchanged test samples of exemplary glass compositions B—F demonstrated lower amounts of extracted Na_2O per gram of glass than the non-ion exchanged test samples of the same exemplary glass compositions following testing according to the ISO 720 standard.

TABLE 8

Composition and Properties of Exemplary Glass Compositions

	Composition in mole %					
	A	B	C	D	E	F
SiO_2	70.8	72.8	74.8	76.8	76.8	77.4
Al_2O_3	7.5	7	6.5	6	6	7
Na_2O	13.7	12.7	11.7	10.7	11.6	10
K_2O	1	1	1	1	0.1	0.1
MgO	6.3	5.8	5.3	4.8	4.8	4.8
CaO	0.5	0.5	0.5	0.5	0.5	0.5
SnO_2	0.2	0.2	0.2	0.2	0.2	0.2
DIN 12116 (mg/dm^2)	3.2	2.0	1.7	1.6	1.2	1.7
classification	S3	S3	S3	S3	S2	S3
ISO 695 (mg/dm^2)	60.7	65.4	77.9	71.5	76.5	62.4
classification	A1	A1	A2	A1	A2	A1
ISO 720 (ug Na_2O/ g glass)	100.7	87.0	54.8	57.5	50.7	37.7
classification	HGA2	HGA2	HGA1	HGA1	HGA1	HGA1
ISO 720 (with IX) (ug Na_2O/ g glass)	60.3	51.9	39.0	30.1	32.9	23.3
classification	HGA1	HGA1	HGA1	HGA1	HGA1	HGA1

Example 2

Three exemplary inventive glass compositions (compositions G-I) and three comparative glass compositions (compositions 1-3) were prepared. The ratio of alkali oxides to alumina (i.e., Y:X) was varied in each of the compositions in order to assess the effect of this ratio on various properties of the resultant glass melt and glass. The specific compositions of each of the exemplary inventive glass compositions and the comparative glass compositions are reported in Table 9. The strain point, anneal point, and softening point of melts formed from each of the glass compositions were determined and are reported in Table 2. In addition, the coefficient of thermal expansion (CTE), density, and stress optic coefficient (SOC) of the resultant glasses were also determined and are reported in Table 9. The hydrolytic resistance of glass samples formed from each exemplary inventive glass composition and each comparative glass composition was determined according to the ISO 720 Standard both before ion exchange and after ion exchange in a molten salt bath of 100% KNO_3 at 450° C. for 5 hours. For those samples that were ion exchanged, the compressive stress was determined with a fundamental stress meter (FSM) instrument, with the compressive stress value based on the measured stress optical coefficient (SOC). The FSM instrument couples light into and out of the birefringent glass surface. The measured birefringence is then related to stress through a material constant, the stress-optic or photoelastic coefficient (SOC or PEC) and two parameters are obtained: the maximum surface compressive stress (CS) and the exchanged depth of layer (DOL). The diffusivity of the alkali ions in the glass and the change in stress per square root of time were also determined

TABLE 9

Glass properties as a function of alkali to alumina ratio

	Composition Mole %					
	G	H	I	1	2	3
SiO_2	76.965	76.852	76.962	76.919	76.960	77.156
Al_2O_3	5.943	6.974	7.958	8.950	4.977	3.997
Na_2O	11.427	10.473	9.451	8.468	12.393	13.277
K_2O	0.101	0.100	0.102	0.105	0.100	0.100
MgO	4.842	4.878	4.802	4.836	4.852	4.757
CaO	0.474	0.478	0.481	0.480	0.468	0.462
SnO_2	0.198	0.195	0.197	0.197	0.196	0.196
Strain (° C.)	578	616	654	683	548	518
Anneal (° C.)	633	674	716	745	600	567
Softening (° C.)	892	946	1003	1042	846	798
Expansion (10^{-7} K^{-1})	67.3	64.3	59.3	55.1	71.8	74.6
Density (g/cm^3)	2.388	2.384	2.381	2.382	2.392	2.396
SOC (nm/mm/Mpa)	3.127	3.181	3.195	3.232	3.066	3.038
ISO720 (non-IX)	88.4	60.9	47.3	38.4	117.1	208.1
ISO720 (IX450° C.-5 hr)	25.3	26	20.5	17.8	57.5	102.5
R_2O/Al_2O_3	1.940	1.516	1.200	0.958	2.510	3.347
CS@t = 0 (MPa)	708	743	738	655	623	502
CS/√t ($MPa/hr^{1/2}$)	−35	−24	−14	−7	−44	−37
D ($\mu m^2/hr$)	52.0	53.2	50.3	45.1	51.1	52.4

The data in Table 9 indicates that the alkali to alumina ratio Y:X influences the melting behavior, hydrolytic resistance, and the compressive stress obtainable through ion exchange strengthening. In particular, FIG. **1** graphically depicts the strain point, anneal point, and softening point as a function of Y:X ratio for the glass compositions of Table 9. FIG. **1** demonstrates that, as the ratio of Y:X decreases below 0.9, the strain point, anneal point, and softening point of the glass rapidly increase. Accordingly, to obtain a glass which is readily meltable and formable, the ratio Y:X should be greater than or equal to 0.9 or even greater than or equal to 1.

Further, the data in Table 2 indicates that the diffusivity of the glass compositions generally decreases with the ratio of Y:X. Accordingly, to achieve glasses can be rapidly ion exchanged in order to reduce process times (and costs) the ratio of Y:X should be greater than or equal to 0.9 or even greater than or equal to 1.

Moreover, FIG. **2** indicates that for a given ion exchange time and ion exchange temperature, the maximum compressive stresses are obtained when the ratio of Y:X is greater than or equal to about 0.9, or even greater than or equal to about 1, and less than or equal to about 2, specifically greater than or equal to about 1.3 and less than or equal to about 2.0. Accordingly, the maximum improvement in the load bearing strength of the glass can be obtained when the ratio of Y:X is greater than about 1 and less than or equal to about 2. It is generally understood that the maximum stress achievable by ion exchange will decay with increasing ion-exchange duration as indicated by the stress change rate (i.e., the measured compressive stress divided by the square root of the ion exchange time). FIG. **2** generally shows that the stress change rate decreases as the ratio Y:X decreases.

FIG. **3** graphically depicts the hydrolytic resistance (y-axis) as a function of the ratio Y:X α-axis). As shown in FIG. **3**, the hydrolytic resistance of the glasses generally improves as the ratio Y:X decreases.

Based on the foregoing it should be understood that glasses with good melt behavior, superior ion exchange performance, and superior hydrolytic resistance can be achieved by maintaining the ratio Y:X in the glass from greater than or equal to about 0.9, or even greater than or equal to about 1, and less than or equal to about 2.

Example 3

Three exemplary inventive glass compositions (compositions J-L) and three comparative glass compositions (compositions 4-6) were prepared. The concentration of MgO and CaO in the glass compositions was varied to produce both MgO-rich compositions (i.e., compositions J-L and 4) and CaO-rich compositions (i.e., compositions 5-6). The relative amounts of MgO and CaO were also varied such that the glass compositions had different values for the ratio (CaO/(CaO+MgO)). The specific compositions of each of the exemplary inventive glass compositions and the comparative glass compositions are reported below in Table 10. The properties of each composition were determined as described above with respect to Example 2.

TABLE 10

Glass properties as function of CaO content

	Composition Mole %					
	J	K	L	4	5	6
SiO_2	76.99	77.10	77.10	77.01	76.97	77.12
Al_2O_3	5.98	5.97	5.96	5.96	5.97	5.98
Na_2O	11.38	11.33	11.37	11.38	11.40	11.34
K_2O	0.10	0.10	0.10	0.10	0.10	0.10
MgO	5.23	4.79	3.78	2.83	1.84	0.09
CaO	0.07	0.45	1.45	2.46	3.47	5.12
SnO_2	0.20	0.19	0.19	0.19	0.19	0.19
Strain (° C.)	585	579	568	562	566	561
Anneal (° C.)	641	634	620	612	611	610
Softening (° C.)	902	895	872	859	847	834
Expansion (10^{-7} K^{-1})	67.9	67.1	68.1	68.8	69.4	70.1
Density (g/cm^3)	2.384	2.387	2.394	2.402	2.41	2.42
SOC nm/mm/Mpa	3.12	3.08	3.04	3.06	3.04	3.01
ISO720 (non-IX)	83.2	83.9	86	86	88.7	96.9
ISO720 (IX450° C. - 5 hr)	29.1		28.4	33.2	37.3	40.1
Fraction of RO as CaO	0.014	0.086	0.277	0.465	0.654	0.982
CS@t = 0 (MPa)	707	717	713	689	693	676
CS/√t ($MPa/hr^{1/2}$)	−36	−37	−39	−38	−43	−44
D ($\mu m^2/hr$)	57.2	50.8	40.2	31.4	26.4	20.7

FIG. **4** graphically depicts the diffusivity D of the compositions listed in Table 10 as a function of the ratio (CaO/(CaO+MgO)). Specifically, FIG. **4** indicates that as the ratio (CaO/(CaO+MgO)) increases, the diffusivity of alkali ions in the resultant glass decreases thereby diminishing the ion exchange performance of the glass. This trend is supported by the data in Table 10 and FIG. **5**. FIG. **5** graphically depicts the maximum compressive stress and stress change rate (y-axes) as a function of the ratio (CaO/(CaO+MgO)). FIG. **5** indicates that as the ratio (CaO/(CaO+MgO)) increases, the maximum obtainable compressive stress decreases for a given ion exchange temperature and ion exchange time. FIG. **5** also indicates that as the ratio (CaO/(CaO+MgO)) increases, the stress change rate increases (i.e., becomes more negative and less desirable).

Accordingly, based on the data in Table 10 and FIGS. **4** and **5**, it should be understood that glasses with higher diffusivities can be produced by minimizing the ratio (CaO/(CaO+MgO)). It has been determined that glasses with suitable diffusivities can be produced when the (CaO/(CaO+MgO)) ratio is less than about 0.5. The diffusivity values of the glass when the (CaO/(CaO+MgO)) ratio is less than about 0.5 decreases the ion exchange process times needed to achieve a given compressive stress and depth of layer. Alternatively, glasses with higher diffusivities due to the ratio (CaO/(CaO+MgO)) may be used to achieve a higher compressive stress and depth of layer for a given ion exchange temperature and ion exchange time.

Moreover, the data in Table 10 also indicates that decreasing the ratio (CaO/(CaO+MgO)) by increasing the MgO concentration generally improves the resistance of the glass to hydrolytic degradation as measured by the ISO 720 standard.

Example 4

Three exemplary inventive glass compositions (compositions M-O) and three comparative glass compositions (com-

positions 7-9) were prepared. The concentration of B_2O_3 in the glass compositions was varied from 0 mol. % to about 4.6 mol. % such that the resultant glasses had different values for the ratio $B_2O_3/(R_2O—Al_2O_3)$. The specific compositions of each of the exemplary inventive glass compositions and the comparative glass compositions are reported below in Table 11. The properties of each glass composition were determined as described above with respect to Examples 2 and 3.

TABLE 11

Glass properties as a function of B_2O_3 content

	Composition Mole %					
	M	N	O	7	8	9
SiO_2	76.860	76.778	76.396	74.780	73.843	72.782
Al_2O_3	5.964	5.948	5.919	5.793	5.720	5.867
B_2O_3	0.000	0.214	0.777	2.840	4.443	4.636
Na_2O	11.486	11.408	11.294	11.036	10.580	11.099
K_2O	0.101	0.100	0.100	0.098	0.088	0.098
MgO	4.849	4.827	4.801	4.754	4.645	4.817
CaO	0.492	0.480	0.475	0.463	0.453	0.465
SnO_2	0.197	0.192	0.192	0.188	0.183	0.189
Strain (° C.)	579	575	572	560	552	548
Anneal (° C.)	632	626	622	606	597	590
Softening (° C.)	889	880	873	836	816	801
Expansion (10^{-7} K^{-1})	68.3	67.4	67.4	65.8	64.1	67.3
Density (g/cm^3)	2.388	2.389	2.390	2.394	2.392	2.403
SOC (nm/mm/MPa)	3.13	3.12	3.13	3.17	3.21	3.18
ISO720 (non-IX)	86.3	78.8	68.5	64.4	52.7	54.1
ISO720 (IX450° C.-5 hr)	32.2	30.1	26	24.7	22.6	26.7
$B_2O_3/(R_2O - Al_2O_3)$	0.000	0.038	0.142	0.532	0.898	0.870
CS@t = 0 (MPa)	703	714	722	701	686	734
CS/√t ($MPa/hr^{1/2}$)	−38	−38	−38	−33	−32	−39
D ($\mu m^2/hr$)	51.7	43.8	38.6	22.9	16.6	15.6

FIG. **6** graphically depicts the diffusivity D (y-axis) of the glass compositions in Table 11 as a function of the ratio $B_2O_3/(R_2O—Al_2O_3)$ (x-axis) for the glass compositions of Table 11. As shown in FIG. **6**, the diffusivity of alkali ions in the glass generally decreases as the ratio $B_2O_3/(R_2O—Al_2O_3)$ increases.

FIG. **7** graphically depicts the hydrolytic resistance according to the ISO 720 standard (y-axis) as a function of the ratio $B_2O_3/(R_2O—Al_2O_3)$ (x-axis) for the glass compositions of Table 11. As shown in FIG. **6**, the hydrolytic resistance of the glass compositions generally improves as the ratio $B_2O_3/(R_2O—Al_2O_3)$ increases.

Based on FIGS. **6** and **7**, it should be understood that minimizing the ratio $B_2O_3/(R_2O—Al_2O_3)$ improves the diffusivity of alkali ions in the glass thereby improving the ion exchange characteristics of the glass. Further, increasing the ratio $B_2O_3/(R_2O—Al_2O_3)$ also generally improves the resistance of the glass to hydrolytic degradation. In addition, it has been found that the resistance of the glass to degradation in acidic solutions (as measured by the DIN 12116 standard) generally improves with decreasing concentrations of B_2O_3. Accordingly, it has been determined that maintaining the ratio $B_2O_3/(R_2O—Al_2O_3)$ to less than or equal to about 0.3 provides the glass with improved hydrolytic and acid resistances as well as providing for improved ion exchange characteristics.

It should now be understood that the glass compositions described herein exhibit chemical durability as well as mechanical durability following ion exchange. These properties make the glass compositions well suited for use in various applications including, without limitation, pharmaceutical packaging materials.

Example 5: Determining the Presence and Amount of Glass Flakes in Pharmaceutical Solutions

The resistance to delamination may be characterized by the number of glass particulates present in a pharmaceutical solution contained within a glass container described herein after. In order to assess the long-term resistance of the glass container to delamination, an accelerated delamination test is utilized. The test consists of washing the glass container at room temperature for 1 minute and depyrogenating the container at about 320° C. for 1 hour. Thereafter a pharmaceutical solution is placed in the glass container to 80-90% full, the glass container is closed, and rapidly heated to, for example, 100° C. and then heated from 100° C. to 121° C. at a ramp rate of 1 deg/min at a pressure of 2 atmospheres. The glass container and solution are held at this temperature for 60 minutes, cooled to room temperature at a rate of 0.5 deg./min and the heating cycle and hold are repeated. The glass container is then heated to 50° C. and held for two days for elevated temperature conditioning. After heating, the glass container is dropped from a distance of at least 18" onto a firm surface, such as a laminated tile floor, to dislodge any flakes or particles that are weakly adhered to the inner surface of the glass container.

Thereafter, the pharmaceutical solution contained in the glass container is analyzed to determine the number of glass particles present per liter of solution. Specifically, the solution from the glass container is directly poured onto the center of a Millipore Isopore Membrane filter (Millipore #ATTP02500 held in an assembly with parts #AP1002500 and #M000025A0) attached to vacuum suction to draw the solution through the filter within 10-15 seconds. Particulate flakes are then counted by differential interference contrast microscopy (DIC) in the reflection mode as described in "Differential interference contrast (DIC) microscopy and modulation contrast microscopy" from Fundamentals of light microscopy and digital imaging. New York: Wiley-Liss, pp 153-168. The field of view is set to approximately 1.5 mm×1.5 mm and particles larger than 50 microns are counted manually. There are 9 such measurements made in the center of each filter membrane in a 3×3 pattern with no overlap between images. A minimum of 100 mL of solution is tested. As such, the solution from a plurality of small containers may be pooled to bring the total amount of solution to 100 mL. If the containers contain more than 10 mL of solution, the entire amount of solution from the container is examined for the presence of particles. For containers having a volume greater than 10 mL containers, the test is repeated for a trial of 10 containers formed from the same glass composition under the same processing conditions and the result of the particle count is averaged for the 10 containers to determine an average particle count. Alternatively, in the case of small containers, the test is repeated for a trial of 10 sets of 10 mL of solution, each of which is analyzed and the particle count averaged over the 10 sets to determine an average particle count. Averaging the particle count over multiple containers accounts for potential variations in the delamination behavior of individual containers.

It should be understood that the aforementioned test is used to identify particles which are shed from the interior wall(s) of the glass container due to delamination and not tramp particles present in the container from forming processes. Specifically, delamination particles will be differentiated from tramp glass particles based on the aspect ratio of the particle (i.e., the ratio of the width of the particle to the thickness of the particle). Delamination produces particulate flakes or lamellae which are irregularly shaped and are typically >50 μm in diameter but often >200 μm. The thickness of the flakes is usually greater than about 100 nm and may be as large as about 1 μm. Thus, the minimum aspect ratio of the flakes is typically >50. The aspect ratio may be greater than 100 and sometimes greater than 1000. Particles resulting from delamination processes generally have an aspect ratio which is generally greater than about 50. In contrast, tramp glass particles will generally have a low aspect ratio which is less than about 3. Accordingly, particles resulting from delamination may be differentiated from tramp particles based on aspect ratio during observation with the microscope. Validation results can be accomplished by evaluating the heel region of the tested containers. Upon observation, evidence of skin corrosion/pitting/flake removal, as described in "Nondestructive Detection of Glass Vial Inner Surface Morphology with Differential Interference Contrast Microscopy" from Journal of Pharmaceutical Sciences 101(4), 2012, pages 1378-1384, is noted.

Using this method, pharmaceutical compositions can be tested for the presence of glass flakes and various compositions can be compared to each other to assess the safety of various pharmaceutical compositions.

Example 6: Stability Testing of Pharmaceutical Compositions

Stability studies are part of the testing required by the FDA and other regulatory agencies. Stability studies should include testing of those attributes of the API that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes of the API (e.g., small molecule or biologic therapeutic agent) in the container with the closure to be used for storage of the agent. If the API is formulated as a liquid by the manufacturer, the final formulation should be assayed for stability. If the API is formulated as an agent for reconstitution by the end user using a solution provided by the manufacturer, both the API and the solution for reconstitution are preferably tested for stability as the separate packaged components (e.g., the API subjected to storage reconstituted with solution for reconstitution not subject to storage, API not subject to storage reconstituted with a solution subject to storage, and both API and solution subject to storage). This is particularly the case when the solution for reconstitution includes an active agent (e.g., an adjuvant for reconstitution of a vaccine).

In general, a substance API should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use.

API should be stored in the container(s) in which the API will be provided to the end user (e.g., vials, ampules, syringes, injectable devices). Stability testing methods provided herein refer to samples being removed from the storage or stress conditions indicated. Removal of a sample preferably refers to removing an entire container from the storage or stress conditions. Removal of a sample should not be understood as withdrawing a portion of the API from the container as removal of a portion of the API from the container would result in changes of fill volume, gas environment, etc. At the time of testing the API subject to stability and/or stress testing, portions of the samples subject to stability and/or stress testing can be used for individual assays.

The long-term testing should cover a minimum of 12 months' duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed retest period. Additional data accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and, if appropriate, from the intermediate storage condition can be used to evaluate the effect of short-term excursions outside the label storage conditions (such as might occur during shipping).

Long-term, accelerated, and, where appropriate, intermediate storage conditions for API are detailed in the sections below. The general case should apply if the API is not specifically covered by a subsequent section. It is understood that the time points for analysis indicated in the table are suggested end points for analysis. Interim analysis can be preformed at shorter time points (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months). For API to be labeled as stable for storage for more than 12 months, time points beyond 12 months can be assessed (e.g., 15, 18, 21, 24 months). Alternative storage conditions can be used if justified.

TABLE 12

General Conditions for Stability Analysis

Study	Storage condition	Time points for analysis
Long-term	Long-term* 25° C. ± 2° C./60% RH ± 5% RH or 30° C. ± 2° C./65% RH ± 5% RH	12 months
Intermediate	30° C. ± 2° C./65% RH ± 5% RH	6 months
Accelerated	40° C. ± 2° C./75% RH ± 5% RH	6 months

TABLE 13

Conditions for Stability Analysis for Storage in a Refrigerator

Study	Storage condition	Minimum time period covered by data at submission
Long-term	5° C. ± 3° C.	12 months
Accelerated	25° C. ± 2° C./60% RH ± 5% RH	6 months

TABLE 14

Conditions for Stability Analysis for Storage in a Freezer

Study	Storage condition	Minimum time period covered by data at submission
Long-term	−20° C. ± 5° C.	12 months

Storage condition for API intended to be stored in a freezer, testing on a single batch at an elevated temperature

(e.g., 5° C.±3° C. or 25° C.±2° C.) for an appropriate time period should be conducted to address the effect of short-term excursions outside the proposed label storage condition (e.g., stress during shipping or handling, e.g., increased temperature, multiple freeze-thaw cycles, storage in a non-upright orientation, shaking, etc.).

The assays performed to assess stability of an API include assays to that are used across most APIs to assess the physical properties of the API, e.g., degradation, pH, color, particulate formation, concentration, toxicity, etc. Assays to detect the general properties of the API are also selected based on the chemical class of the agent, e.g., denaturation and aggregation of protein based API. Assays to detect the potency of the API, i.e., the ability of the API to achieve its intended effect as demonstrated by the quantitative measurement of an attribute indicative of the clinical effect as compared to an appropriate control, are selected based on the activity of the particular agent. For example, the biological activity of the API, e.g., enzyme inhibitor activity, cell killing activity, anti-inflammatory activity, coagulation modulating activity, etc., is measured using in vitro and/or in vivo assays such as those provided herein. Pharmacokinetic and toxicological properties of the API are also assessed using methods known in the art, such as those provided herein.

Example 7: Analysis of Adherence to Glass Vials

Changes in the surface of glass can result in changes in the adherence of API to glass. The amount of agent in samples withdrawn from glass vials are tested at intervals to determine if the concentration of the API in solution changes over time. API are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the API is incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed to determine the concentration of the API in solution. The concentration of the API is determined using methods and controls appropriate to the API. The concentration of the API is preferably determined in conjunction with at least one assay to confirm that the API, rather than degradation products of the API, is detected. In the case of biologics in which the conformational structure of the biologic agent is essential to its function of the API, the assays for concentration of the biologic are preferably preformed in conjunction with an assay to confirm the structure of the biologic (e.g., activity assay).

For example, in the cases of small molecule APIs, the amount of agent present is determined, for example, by mass spectrometry, optionally in combination with liquid chromatography, as appropriate, to separate the agent from any degradation products that may be present in the sample.

For protein based biologic APIs, the concentration of the API is determined, for example, using ELISA assay. Chromatography methods are used in conjunction with methods to determine protein concentration to confirm that protein fragments or aggregates are not being detected by the ELISA assay.

For nucleic acid biologic APIs, the concentration of the API is determined, for example, using quantitative PCR when the nucleic acids are of sufficient length to permit detection by such methods. Chromatography methods are used to determine both the concentration and size of nucleic acid based API.

For viral vaccine APIs, the concentration of the virus is determined, for example, using colony formation assays.

Example 8: Analysis of Pharmacokinetic Properties

Pharmacokinetics is concerned with the analysis of absorption, distribution, metabolism, and excretion of API. Storage and stress can potentially affect the pharmacokinetic properties of various API. To assess pharmacokinetics of API subject to stability and/or stress testing, agents are incubated in containers as described in Example 6. Preferably, the API are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed.

The API is delivered to subjects by the typical route of delivery for the API (e.g., injection, oral, topical). As pharmacokinetics are concerned with the absorption and elimination of the API, normal subjects are typically used to assess pharmacokinetic properties of the API. However, if the API is to be used in subjects with compromised ability to absorb or eliminate the API (e.g., subjects with liver or kidney disease), testing in an appropriate disease model may be advantageous. Depending on the half life of the compound, samples (e.g., blood, urine, stool) are collected at predetermined time points (e.g., 0 min, 30 min, 60 min, 90 min, 120 min, 4 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, etc.) for at least two, preferably three half-lives of the API, and analyzed for the presence of the API and metabolic products of the API. At the end of the study, organs are harvested and analyzed for the presence of the API and metabolic products of the API.

The results are analyzed using an appropriate model selected based on, at least, the route of administration of the API. The pharmacokinetic properties of the API subjected to stability and/or stress testing are compared to API not subjected to stability or stress testing and other appropriate controls (e.g., vehicle control). Changes, if any, in pharmacokinetic properties as a result of storage of the API under each condition are determined.

Example 9: Analysis of Toxicity Profiles

Storage of API can result in alterations of toxicity of API as a result of reactivity of the API with the container, leeching of agents from the container, delamination resulting in particulates in the agent, reaction of the API molecules with each other or components of the storage buffer, or other causes.

Agents are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the API is incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed to determine the toxicity the API. The toxicity of the API is determined using methods and controls appropriate to the API. In vitro and in vivo testing can be used alone or in combination to assess changes in toxicity of agents as a result of storage or stress.

In in vitro assays, cell lines are grown in culture and contacted with increasing concentrations of API subjected to stability and/or stress testing for predetermined amounts of time (e.g., 12, 24, 36, 48, and 72 hours). Cell viability is assessed using any of a number of routine or commercially available assays. Cells are observed, for example, by microscopy or using fluorescence activated cell sorting (FACS) analysis using commercially available reagents and kits. For

example, membrane-permeant calcein AM is cleaved by esterases in live cells to yield cytoplasmic green fluorescence, and membrane-impermeant ethidium homodimer-1 labels nucleic acids of membrane-compromised cells with red fluorescence. Membrane-permeant SYTO 10 dye labels the nucleic acids of live cells with green fluorescence, and membrane-impermeant DEAD Red dye labels nucleic acids of membrane-compromised cells with red fluorescence. A change in the level of cell viability is detected between the cells contacted with API subjected to stress and/or stability testing in standard glass vials as compared to the glass vials provided herein and appropriate controls (e.g., API not subject to stability testing, vehicle control).

In vivo toxicity assays are performed in animals. Typically preliminary assays are performed on normal subjects. However, if the disease or condition to be treated could alter the susceptibility of the subject to toxic agents (e.g., decreased liver function, decreased kidney function), toxicity testing in an appropriate model of the disease or condition can be advantageous. One or more doses of agents subjected to stability and/or stress testing are administered to animals. Typically, doses are far higher (e.g., 5 times, 10 times) the dose that would be used therapeutically and are selected, at least in part, on the toxicity of the API not subject to stability and/or stress testing. However, for the purpose of assaying stability of API, the agent can be administered at a single dose that is close to (e.g., 70%-90%), but not at, a dose that would be toxic for the API not subject to stability or stress testing. In single dose studies, after administration of the API subject to stress and/or stability testing (e.g., 12 hours, 24 hours, 48 hours, 72 hours), during which time blood, urine, and stool samples may be collected. In long term studies, animals are administered a lower dose, closer to the dose used for therapeutic treatment, and are observed for changes indicating toxicity, e.g., weight loss, loss of appetite, physical changes, or death. In both short and long term studies, organs are harvested and analyzed to determine if the API is toxic. Organs of most interest are those involved in clearance of the API, e.g., liver and kidneys, and those for which toxicity would be most catastrophic, e.g., heart, brain. An analysis is performed to detect a change in toxicity between the API subjected to stress and/or stability testing in standard glass vials as compared to the glass vials provided herein, as compared to API not subject to stability and/or stress testing and vehicle control. Changes, if any, in toxicity properties as a result of storage of the API under each condition are determined

Example 10: Analysis of Pharmacodynamic Profiles

Pharmacodynamics includes the study of the biochemical and physiological effects of drugs on the body or on microorganisms or parasites within or on the body and the mechanisms of drug action and the relationship between drug concentration and effect. Mouse models for a large variety of disease states are known and commercially available (see, e.g., jaxmice.jax.org/query/f?p=205:1:989373419139701::::P1_ADV:1). A number of induced models of disease are also known.

Agents are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the samples are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed for pharmacodynamic activity using known animal models. Exemplary mouse models for testing the various classes of agents indicated are known in the art.

The mouse is treated with the API subject to stability and/or stress testing. The efficacy of the API subject to stability and/or stress testing to treat the appropriate disease or condition is assayed as compared to API not subject to stability and/or stress testing and vehicle control. Changes, if any, in pharmacodynamic properties as a result of storage of the API under each condition are determined.

Example 11: Confirmation of Stability and Integrity of Protein API by Size Determination

A number of protein and peptide-based API are known. Upon storage, proteins can denature, forming disordered higher order structures, e.g., aggregates. Conversely, proteins with higher order, multimeric structures can be disassemble into lower order structures through loss of covalent (disulfide) or non-covalent interactions. Proteins can also degrade over time under stress or even normal storage conditions.

Size can be used as preliminary indicator of stability of a protein API that has undergone stability and/or stress testing. Assays to detect the size and subunit structure of a protein API can be broadly applied to any of a number of API. Exemplary assays are provided. Other methods known in the art can also be used.

Agents are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the samples are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed to determine API size and subunit structure. Exemplary methods for determining protein size and subunit structure are provided below. Other methods are well known in the art.

Chromatography Methods for Size Detection

Samples of protein API subject or not subject to stability and/or stress testing are diluted and subject to analysis by size exclusion chromatography (SEC), preferably SEC-high performance liquid chromatography (SEC-HPLC). Chromatography columns are available from a number of commercial vendors (e.g., WATERS, BIO-RAD). Size exclusion chromatography columns separate compounds by gel filtration chromatography. The technique is based on diffusion in and around highly porous spherical silica beads. The degree of retention depends on the size and shape of the solute molecule and the pore size of the packing. Large molecules are sterically excluded and pass around the beads, eluting from the column before smaller molecules, which totally or partially permeate the material and are eluted later. The working range of the gel is the volume between the void volume, those solutes which elute first, and the totally included volume, those solutes which elute last. Protein aggregates; complexes, e.g., dimers; monomers; and fragments, can be detected by photometry for absorbance at 350-500 nm or by UV detection at 280 nm.

The specific column type, column size, solvent type, flow rate, and detector type can be selected based on the specific API to be analyzed. Different solvents can be selected to detect monomer or higher order structures. Such considerations are well understood by those of skill in the art.

The relative amounts of aggregates, dimers, monomers, and/or degradation products are determined in the sample that has undergone stability and/or stress testing and compared to the control sample that has not undergone stability

and/or stress testing. The total protein concentration is also determined. A change in the amount of aggregate, dimer, monomer, or degradation products; and/or a change in total protein concentration, indicate a change in the API as a result of stress and/or stability testing.

Gel Electrophoresis Methods for Size Detection

Samples of protein API subject or not subject to stability and/or stress testing are diluted and resolved by gel electrophoresis. SDS-PAGE is used to determine protein size and aggregate formation. Native gel electrophoresis is used to analyze protein complexes formed by covalent or non-covalent interaction. Non-reducing gel electrophoresis is used to analyze protein complexes or protein structures containing disulfide bonds. Proteins resolved by gel electrophoresis are detected using sensitive staining methods, such as silver stain. Gel electrophoresis and silver staining methods are known in the art. A change in the amount of aggregate, dimer, monomer, or degradation products; and/or a change in total protein concentration, indicate a change in the API as a result of stress and/or stability testing.

Example 12: Confirmation of Stability and Integrity of Protein API by Antibody Binding

A number of protein and peptide-based API are known. Protein concentration, and sometimes protein conformation, can be assessed using an enzyme-linked immuno sorbent assay (ELISA).

Agents are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the samples are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed using ELISA. A sandwich ELISA method is described briefly herein. Variations based on the specific API, antibody, and ELISA format used, as well as selection of appropriate dilutions and controls, are well within the ability of those of skill in the art.

In sandwich ELISA methods for the detection of a protein API, a first antibody that binds the API is bound to a solid support, e.g., a 96- or 384-well plate. Samples of protein API subject or not subject to stability and/or stress testing are diluted and contacted with the first antibody attached to the solid support. The complex is contacted with a second antibody that binds to the API at an epitope distinct from the epitope bound by the first antibody. Therefore, the amount of the second antibody present in the well is dependent upon the amount of API in the well. If desired, one or both of the antibodies used can bind a conformational epitope on the API that can detect if the API is properly folded. Alternatively, if the API is prone to cleavage at a particular site, the one antibody can bind to a portion of the API N-terminal to the cleavage site, and the other antibody can bind to a portion of the API C-terminal to the cleavage site so that only full length API is detected. Further, if the API is a heterodimer, the first antibody can bind to the first peptide of the heterodimer, and the second antibody can bind to the second peptide of the heterodimer so that only heterodimers are detected. Other variations can be conceived by those of skill in the art.

The second antibody bound to the API is detected using a detectable label. The detectable label is typically an enzyme (e.g., alkaline phosphatase, horseradish peroxidase) that is bound to a third antibody that specifically binds the second antibody bound to the API. The amount of API detected samples subject or not subject to stability and/or stress testing are compared. Depending on the specific antibodies used, changes in the concentration of the API, and possibly the structure of the API, can be detected. Changes, if any, in the results from the ELISA between the samples subject or not subject to stress and/or stability testing are noted.

Example 13: Confirmation of Stability and Activity of GAMMAGARD Liquid or GAMMAGARD S/D (Immune Globulin, Human)

GAMMAGARD LIQUID is a ready-for-use sterile, liquid preparation of highly purified and concentrated immunoglobulin G (IgG) antibodies. The distribution of the IgG subclasses is similar to that of normal plasma. The Fc and Fab functions are maintained in GAMMAGARD LIQUID or GAMMAGARD S/D. Pre-kallikrein activator activity is not detectable. GAMMAGARD LIQUID or GAMMAGARD S/D contains 100 milligram/mL protein. At least 98% of the protein is immune globulin, the average immunoglobulin A (IgA) concentration is 37 μg/mL, and immunoglobulin M is present in trace amounts. GAMMAGARD LIQUID or GAMMAGARD S/D contains a broad spectrum of IgG antibodies against bacterial and viral agents. Glycine (0.25M) serves as a stabilizing and buffering agent, and there are no added sugars, sodium or preservatives. The pH is 4.6 to 5.1. The osmolality is 240 to 300 mOsmol/kg, which is similar to physiological osmolality (285 to 295 mOsmol/kg). Activity of immunoglobulin can be tested using known methods in the art to assay protein size and structure and pharmacokinetic properties such as those provided herein.

GAMMAGARD LIQUID or GAMMAGARD S/D is incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the samples are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed to determine stability and/or activity of the agent. The activity of GAMMAGARD LIQUID or GAMMAGARD S/D is determined using methods and controls appropriate for the agent, e.g., using the methods provided in US Patent Publication No. US20070122402, and the methods provided below.

Size Detection to Confirm Stability and Detect Aggregate Formation in GAMMAGARD LIQUID or GAMMAGARD S/D

Samples of GAMMAGARD LIQUID or GAMMAGARD S/D subject or not subject to stability and/or stress testing, e.g., using the methods in Example 6, are diluted and subject to analysis by size exclusion chromatograph, e.g., using a Waters HPLC system with a BIOSEP SEC 3000 column, to detect aggregates, dimeric, monomeric IgG and fragments, by photometry for absorbance at 350-500 nm. The relative amounts of aggregates, dimers, monomers, and degradation products are determined and compared to a control sample that has not undergone stability and/or stress testing. The total protein concentration is also determined. Relative amounts of aggregates, dimers, monomers, and degradation products, as well as the concentration of protein in the sample that has undergone stability and/or stress testing is compared to the control sample. The amount of aggregate, dimer, degradation product, or total protein are determined. Changes, if any, in the amount of aggregate, dimer, degradation product, or total protein between the samples subject or not subject to stress and/or stability testing are noted.

Alternatively, or in conjunction with chromatography methods, gel electrophoresis under reducing and/or non-

reducing conditions are used in combination with silver staining to assess the amount of aggregate, dimer, degradation product, or total protein present in each sample. Changes, if any, in the amount of aggregate, dimer, degradation product, or total protein between the samples subject or not subject to stress and/or stability testing are noted.

Pharmacokinetic Analysis

GAMMAGARD LIQUID or GAMMAGARD S/D is an immune globulin (human) indicated as replacement therapy for primary humoral immunodeficiency (PI). A number of immunodeficient mouse strains are known in the art (see, e.g., Choosing an Immunodeficient Mouse Model, JAX® NOTES Issue 501, Spring 2006, available on the world wide web at jaxmice.jax.org/jaxnotes/archive/501a.html). A mouse strain that will not have an immune response to GAMMAGARD LIQUID or GAMMAGARD S/D is used to assay changes as a result of stability and/or stress testing. Samples of GAMMAGARD LIQUID or GAMMAGARD S/D subject or not subject to stability and/or stress testing are used to analyze the pharmacokinetic properties of the samples. Blood samples for pharmacokinetic assessment are taken before the infusion and at predetermined times after the infusion, e.g., 15 minutes, 1 hour, 24 hours, 3 days, 7 days, 14 days, 21 days, and 28 days. The samples are tested for concentrations of total human IgG and IgG subclasses using routine methods (e.g., ELISA). The calculated pharmacokinetic parameters can include C_{max}, the maximum serum concentration, T_{max}, the time to reach the maximum serum concentration, AUC 0-t, the area under the concentration-time curve over 1 dosing interval, $t_{1/2}$ or the elimination half-life, CL the total body clearance and Vz, the volume of distribution.

Following intravenous infusion, immunoglobulin products show a biphasic decay curve. The initial (a) phase is characterized by an immediate post-infusion peak in serum IgG, and is followed by rapid decay due to equilibration between the plasma and extravascular fluid compartments. The second (b) phase is characterized by a slower and constant rate of decay. The commonly cited "normal" half-life of 18 to 25 days in humans is based on studies in which tiny quantities of radiolabeled IgG are injected into healthy human individuals. Extended half-lives are typically observed in immunocompromised human subjects, and may be observed in immunocompromised mice.

The calculated pharmacokinetic parameters are compared between the samples subject or not subject to stability and/or stress testing. The exact pharmacokinetic properties observed are less critical than the consistency between the samples subject or not subject to stability and/or stress testing. Changes, if any, in pharmacokinetic properties between the samples subject or not subject to stability and/or stress testing are noted.

Example 14: Confirmation of Stability and Activity of ADVATE®(Recombinant Coagulation Factor VIII, rAHF)

ADVATE is a recombinant immune-affinity purified factor VIII protein for use in the treatment of hemophilia A. ADVATE (human Antihemophilic Factor (Recombinant) (rAHF)) is formulated as a sterile, non-pyrogenic, white to off-white, lyophilised powder preparation of recombinant antihaemophilic Factor VIII. It is produced from a genetically engineered Chinese Hamster Ovary (CHO) cell-line MCB 9710 under conditions, which are free from the use of animal derived protein. ADVATE (rAHF) is presented in a glass vial accompanied by sterile Water for Injection (5 mL) for reconstitution (see Dosage and Administration). The reconstituted product is a clear, colourless solution for intravenous (IV) injection. The chemical structure of rAHF is that of a dimeric glycoprotein, which has been shown to have a similar amino acid sequence with that of the human plasma derived factor VIII. Amino acid analysis of the purified glycosylated protein demonstrated that it constitutes 2332 amino acids with a molecular mass of approximately 280 kDa. Thus, the rAHF is a full length factor VIII. The specific activity is approximately 4,000-10,0001 U/mg protein. Activity of factor VIII can be determined using any of a number of routine methods in the art such as the unit activity assay and in vivo clotting assay provided herein.

Samples of rAHF are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the samples are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed to determine stability and/or activity of the agent.

Unit Activity Testing

The number of units of factor VIII in a vial is expressed in International Units (IU), which are related to the current WHO standard for factor VIII products. One International Unit (IU) of factor VIII activity is equivalent to that quantity of factor VIII in one mL of normal human plasma. The potency (IU) is determined using the one-stage clotting assay or by chromogenic method (EP), against a standard that is referenced to the FDA/US Mega I Standard. The standards can be further calibrated against the third WHO standard.

A 1-stage activated partial thromboplastin time (APTT)-based factor assay is widely used for the measurement of factors VIII, IX, X and XI and are well known in the art. Samples of rAHF subject or not subject to stability and/or stress testing are used to analyze the activity level of the protein. Commercially available plasma completely deficient in a factor VIII is used for the APTT assay. Serial dilutions of rAHF subject or not subject to stability and/or stress testing are mixed with an equal volume of substrate plasma deficient in factor VIII and clotting times are determined. Units of activity determined are compared to the number of units of activity expected based on the known amount of product in the vial and between the samples of rAHF subject or not subject to stability and/or stress testing. Changes, if any, in the expected number of units detected are noted.

In Vivo Clotting Assay

Hemophilic mice (5/sex/group) each receive a single intravenous administration of rAHF subject or not subject to stability and/or stress testing, or an equivalent volume of the corresponding formulation vehicles (controls). The mice are then anesthetized and their tails transected approximately 20 mm from the tip. Blood from the transected tails is collected over 20 minutes beginning 30 minutes postdosing and the volume of blood loss as a function of time is analysed.

The mean blood loss observed in the treatment groups over the 20 minutes observation period is significantly lower than the formulation vehicle control groups. Relative blood loss in the mice treated with rAHF subject or not subject to stability and/or stress testing is indicative of the stability of rAHF under testing conditions. Relative activity levels are indicative of the stability of the rAHF under various storage conditions. Changes, if any, in the clotting rate are noted.

Example 15: Confirmation of Stability and Activity of BAX111 (Recombinant Von Willebrand Factor (rVWF))

Bax 111 recombinant von Willebrand factor (rVWF) concentrate (vonicog alfa) is prepared using a plasma- and albumin-free manufacturing method. It is the first recombinant replacement protein in clinical development for von Willebrand disease (VWD). Von Willebrand disease is the most common inherited bleeding disorder and affects both men and women. Patients with VWD either produce insufficient VWF or defective VWF that result in problems with forming clots to stop bleeding, particularly in the skin and mucous membranes such as in the gastrointestinal (GI) tract. It is estimated that up to one percent of the worlds population suffers from von Willebrand disease, but because many people have only mild symptoms, they may not know they have the condition. Research has suggested that as many as nine out of 10 people with VWD have not been diagnosed.

Methods to assay VWF activity are provided, for example, in US Patent Publication No. 20060003921 and Flood et al., 2011 (Gain-of-function GPIb ELISA assay for VWF activity in the Zimmerman Program for Molecular and Biology of VWF, *Blood.* 117:e67-e74), both of which are incorporated herein by reference. Although methods of detection of VWF protein and activity levels in subjects suffering from VWD can provide inconsistent results depending on the assay used, these variations are due, in part, to the various etiologies of VFD. However, as the assays used in relation to the instant invention are to compare the activity of purified, recombinant VWF in identical plasma samples, the absolute values obtained in the assays are less important than any changes observed in VWF protein and activity levels in samples subject or not subject to stability and/or stress testing.

Samples of vonicog alfa are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the samples are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed to determine von Willebrand factor activity. Other methods are well known in the art.

Vonicog Alfa Thrombin Generation Assay in Plasma in the Presence of Platelets

Severe VWD plasma (George King Bio-Medical Inc., USA) is reconstituted with 1 U/ml FVIIIC (Recombinate, Baxter, USA) and incubated with washed platelets from a severe VWD patient (Type III) in the presence of factor eight inhibitor bypass activity (FEIBA) (Immuno, Austria) and $CaCl_2$. Alternatively FVIIa can be used as an activator. Dilute vonicog alfa in samples subject or not subject to stability and/or stress testing are added to the factor VIII reconstituted plasma samples further reconstituted with ristocetin, and thrombin generation is followed. (1 Risto U/ml=the ristocetin cofactor activity of the VWF in normal plasma). Subsamples of the reaction mixture are withdrawn at time intervals and added to a chromogenic substrate for thrombin (D-cyclohexyl-gly-L-Ala-L-Arg-pNA; Immuno, Austria) containing EDTA to stop any further clotting reactions. The chromogenic reaction is stopped by the addition of acetic acid, and the absorbance, which is the function of the thrombin concentration, is measured at 405 nm. Thrombin generation stimulated by vonicog alfa in samples subject or not subject to stability and/or stress testing is compared to detect changes, if any, in activity levels in response to stability and/or stress testing.

VWF:IbCo ELISA to Assay Binding of VWF to GPIbα

A gain-of-function VWF-glycoprotien Ib (GPIbα) construct (tGPIbα 235Y; 239V) containing 2 mutations, D235Y and M239V, with a histidine tag for purification and a C65A mutation to prevent dimerization is used to detect the activity of VWF in an indirect ELISA assay. The D235Y and M239V mutations have been demonstrated to lead to increased VWF binding. The construct extends from nucleotide 85 through 960 (amino acid 290). A similar construct with the wild-type GPIbα sequence is also used. The tGPIbα 235Y; 239V construct can be expressed in S2 insect cells, and supernatant is collected and purified over a nickel column (GE Healthcare). To assay for VWF activity, a monoclonal antibody against human GPIbα is bound to a 96-well enzyme-linked immunosorbent assay (ELISA) plate (Immulon 4 HBX, ThermoScientific) and incubated under appropriate conditions. The tGPIbα 235Y; 239V protein is then added to the wells and incubated under appropriate conditions. Dilute vonicog alfa in samples subject or not subject to stability and/or stress testing are added to the wells and incubated under appropriate conditions. No ristocetin is used in this assay. A mixture of biotinylated monoclonal anti-VWF antibodies (AVW-1 and AVW-15) is used to detect the presence of VWF. Streptavidin-conjugated alkaline phosphatase and p-nitrophenyl phosphate is added and the optical density measured on an ELISA reader.

Lyophilized, reconstituted normal control plasma calibrated to the World Health Organization standard is used to construct the reference curve and compared to the amount of vonicog alfa detected in vonicog alfa in samples subject or not subject to stability and/or stress testing. Vonicog alfa amounts in samples subject or not subject to stability and/or stress testing are compared to detect changes in activity levels, if any, in response to stability and/or stress testing.

In Vivo Clotting Assay

Hemophilic mice (5/sex/group) lacking expression of VWF each receive a single intravenous administration of vonicog alfa subject or not subject to stability and/or stress testing, or an equivalent volume of the corresponding formulation vehicles (controls). The mice are then anesthetized and their tails transected approximately 20 mm from the tip. Blood from the transected tails is collected over 20 minutes beginning 30 minutes postdosing and the volume of blood loss as a function of time is analysed.

The mean blood loss observed in the treatment groups over the 20 minutes observation period is significantly lower than the formulation vehicle control groups. Relative blood loss in the mice treated with vonicog alfa subject or not subject to stability and/or stress testing is indicative of the stability of vonicog alfa under testing conditions.

Example 16: Confirmation of Stability and Activity of an Adenovirus-Associated Viral Vector for Treatment of Hemophilia

The self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette sscAAV2/8-LP1-hFIXco for gene transfer in the treatment of hemophilia B has been described (Nathwani, et al., 2006. Self complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. *Blood.* 107:2653-61; Nathwani, et al., 2007. Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates. *Blood.* 109:1414-21, both of which are

incorporated herein by reference). Exemplary methods for determining scAAV2/8-LP1-hFIXco stability and activity including assays for viral vector titer and determination of transduction efficiency and biodistribution a are provided below. Other methods are well known in the art.

Samples of sscAAV2/8-LP1-hFIXco are incubated in containers as described in the stability testing and/or stress testing methods provided in Example 6. Preferably, the samples are incubated both in standard glass vials with appropriate closures and glass vials such as those provided herein. At the desired intervals, samples are removed and assayed to determine sscAAV2/8-LP1-hFIXco stability and activity.

Analysis of Viral Titer and Nucleic Acid Stability of sscAAV2/8-LP1-hFIX

Vector genome titers are determined by quantitative slot-blot method using supercoiled plasmid DNA as standards as described in Nathwani et al., 2001 (Factors influencing in-vivo transduction by recombinant adeno-associated viral vectors expressing the human factor IX cDNA. *Blood.* 97:1258-1265, incorporated herein by reference). Of importance, each scAAV particle is calculated as containing 2 copies of single stranded viral genomes. Further, the purified vector stocks are confirmed to be free of contamination with wt-AAV and cellular and adenoviral proteins using PCR. To determine the molecular configuration of scAAV, vector stock is incubated with an equal volume of sample loading buffer (60 mM NaOH, 2 mm EDTA, 20% Ficoll, and 0.06% Bromocresol green) and separated on a 1% agarose gel containing 30 mM NaOH and 1 mM EDTA, transferred to nitrocellulose by Southern blotting, and hybridized with a ^{32}P-labeled 424-bp BstApI fragment from the scAAV-LP1-FIXco at 42° C. The intensity of the hybridization is determined using a quantitative method, e.g., using a phosphorimager. The size and intensity of bands in both the slot blot and Southern blot analysis are compared between samples of sscAAV2/8-LP1-hFIX subject to or not subject to stress and/or stability testing to detect changes as a result of the stress and/or stability testing.

Administration of AAV Vectors to Animals for In Vivo Studies

Mice with a disruption in the factor 1× gene, e.g., the HB mouse strain, based on 129/sv mice with disruption of the FIX gene is used for studies to determine transduction efficiency and vector biodistribution. Tail-vein administration of rAAV vector particles is performed in 7- to 10-week-old male mice as described in Nathwani et al., 2001. A two-thirds partial hepatectomy is performed at 16 weeks after tail-vein administration of 1×10^{10} scAAV2/8-LP1-hFIXco using methods known in the art (see, e.g., Nakai et al. 2001, Extrachromosomal recombinant adeno-associated virus vector genomes are primarily responsible for stable liver transduction in vivo. *J. Virol.* 75: 6969-6976, incorporated herein by reference). Four weeks later, at a time when the liver mass is fully reconstituted, the mice are killed to harvest the liver.

Captive bred Macaca mulatta aged between about 2.5 to about 6.5 years and weighing between about 1.5 and 7.5 kg are injected with vector particles in phosphate-buffered saline (PBS) infused into the mesenteric circulation of the macaques using methods known in the art (Nathwani et al. 2002. Sustained high-level expression of human factor IX (hFIX) after liver-targeted delivery of recombinant adeno-associated virus encoding the hFIX gene in rhesus macaques. *Blood.* 100: 1662-1669). The complete blood count (CBC), serum chemistry, and coagulation profile are performed by using routine clinical laboratory methods. Serum IL-6 levels are assessed using an IL-6 immunoassay kit per the manufacturer's instruction. To eradicate anti-hIX antibody in M1-sc treatment with rituximab (250 mg/m^2, 2 doses at 3 weekly intervals) and daily oral cyclosporine (30-100 mg/kg per day, adjusted to maintain through therapeutic levels at 100-500 ng/mL) is started approximately 23 weeks after gene transfer and maintained for a period of 4 weeks.

Determination of Transduction Efficiency and Vector Biodistribution

Human factor IX antigen levels in murine and rhesus samples are determined by enzyme-linked immunosorbent assay (ELISA) according to the previously described methods (Nathwani et al., 2002). The probability of statistical difference between experimental groups is determined by one-way ANOVA and paired student t-test using commercially available software, e.g., GraphPad PRISM version 4.0 software. A one-stage assay for hFIX:C is performed using known methods (see, e.g., Waddington et al., 2004. Permanent phenotypic correction of hemophilia B in immunocompetent mice by prenatal gene therapy. *Blood.* 104: 2714-2721, incorporated herein by reference.) Background hFIX:C levels in untreated hemophiliac HB mice are typically less than 0.03 U/mL. The baseline clotting time of citrated plasma (diluted×100) from untreated HB mice are determined. Thrombin-antithrombin levels are determined using commercially available kits, e.g., the Enzygnost TAT kit (Dade-Behring, Milton Keynes, United Kingdom).

Low-molecular-weight extrachromosomal Hirt DNA is extracted from liver at varying time points after tail-vein administration of scAAV-2/8 vector using previously described methods (Davidoff et al., 2003. Sex significantly influences transduction of murine liver by recombinant adeno-associated viral vectors through an androgen-dependent pathway. *Blood.* 102:480-488, incorporated herein by reference). Undigested Hirt DNA (10 μg) or DNA digested with PstI (which cuts once within the FIX expression cassette) is separated on 1% agarose gel, transferred to nitrocellulose by Southern blotting, and hybridized with an α^{32}P-labeled 842-bp BstApI LP1 fragment at 42° C. The intensity of the hybridization is determined using a phosphorimager and quantitated using commercially available software, e.g., ImageQuant® software. To determine AAV transgene copy number, high-molecular-weight genomic DNA (10 μg) is extracted from murine and macaque tissue samples, using routine methods, digested with either BsrDI or a combination of EcoRI and PstI and electrophoresed through a 0.8% agarose gel for transfer to a nylon membrane. The membrane is then hybridized with the probe and quantitated as described earlier in this section.

To evaluate the biodistribution of AAV-FIX vectors, genomic DNA extracted from various murine and macaque tissues is subjected to PCR using primers that amplified a 617-bp region of hFIXco (5' primer: 5' TTTCCTGATGTGGACTATGT 3' (SEQ ID NO: 4) and 3' primer: 5' TCATGGAAGCCAGCACAGAACATG 3' (SEQ ID NO: 5)) as described previously (Nathwani, 2002). Integrity of DNA is determined by amplifying a 604-bp region of the murine or rhesus β-actin gene using routine methods (see, e.g., Nathwani, 2001). To determine which organs expressed hFIXco, total RNA is isolated and subjected to the reverse-transcription (RT) conditions in the presence or absence of reverse transcriptase using routine methods (see, e.g., Nathwani, 2001). Human FIX expression in various formalin-fixed, paraffin-embedded tissue specimens is analyzed by immunohistochemistry using routine methods (see, e.g., Davidoff et al., 2005. Comparison of the ability of adeno-associated

viral vectors pseudotyped with serotype 2, 5 and 8 capsid proteins to mediate efficient transduction of the liver in murine and nonhuman primate models. *Mol. Ther.* 11:875-888, incorporated herein by reference).

It will be apparent to those skilled in the art that various modifications and variations can be made to the embodiments described herein without departing from the spirit and scope of the claimed subject matter. Thus it is intended that the specification cover the modifications and variations of the various embodiments described herein provided such modification and variations come within the scope of the appended claims and their equivalents.

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Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro
        35                  40                  45

Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys
    50                  55                  60

Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys
65                  70                  75                  80

Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr
                85                  90                  95

Asp His Val Cys Asp Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr
            100                 105                 110

Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr
        115                 120                 125

Val Leu Val Gln Asp Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile
    130                 135                 140

Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys
145                 150                 155                 160

Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly
                165                 170                 175

Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val
            180                 185                 190

Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser
        195                 200                 205

Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr
    210                 215                 220

Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln
225                 230                 235                 240

Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val
                245                 250                 255

Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg
            260                 265                 270

Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met
        275                 280                 285

Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val
    290                 295                 300

Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val
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305                 310                 315                 320

Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys
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Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly
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Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu
        355                 360                 365

Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn
    370                 375                 380

Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu
385                 390                 395                 400

Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro
                405                 410                 415

Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp
            420                 425                 430

Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys
        435                 440                 445

Val Thr Leu Asn Pro Ser Asp Pro Glu His Cys Gln Ile Cys His Cys
    450                 455                 460

Asp Val Val Asn Leu Thr Cys Glu Ala Cys Gln Glu Pro Gly Gly Leu
465                 470                 475                 480

Val Val Pro Pro Thr Asp Ala Pro Val Ser Pro Thr Thr Leu Tyr Val
                485                 490                 495

Glu Asp Ile Ser Glu Pro Pro Leu His Asp Phe Tyr Cys Ser Arg Leu
            500                 505                 510

Leu Asp Leu Val Phe Leu Leu Asp Gly Ser Ser Arg Leu Ser Glu Ala
        515                 520                 525

Glu Phe Glu Val Leu Lys Ala Phe Val Val Asp Met Met Glu Arg Leu
    530                 535                 540

Arg Ile Ser Gln Lys Trp Val Arg Val Ala Val Val Glu Tyr His Asp
545                 550                 555                 560

Gly Ser His Ala Tyr Ile Gly Leu Lys Asp Arg Lys Arg Pro Ser Glu
                565                 570                 575

Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly Ser Gln Val Ala
            580                 585                 590

Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe Gln Ile Phe Ser Lys
        595                 600                 605

Ile Asp Arg Pro Glu Ala Ser Arg Ile Thr Leu Leu Leu Met Ala Ser
    610                 615                 620

Gln Glu Pro Gln Arg Met Ser Arg Asn Phe Val Arg Tyr Val Gln Gly
625                 630                 635                 640

Leu Lys Lys Lys Lys Val Ile Val Ile Pro Val Gly Ile Gly Pro His
                645                 650                 655

Ala Asn Leu Lys Gln Ile Arg Leu Ile Glu Lys Gln Ala Pro Glu Asn
            660                 665                 670

Lys Ala Phe Val Leu Ser Ser Val Asp Glu Leu Glu Gln Gln Arg Asp
        675                 680                 685

Glu Ile Val Ser Tyr Leu Cys Asp Leu Ala Pro Glu Ala Pro Pro Pro
    690                 695                 700

Thr Leu Pro Pro Asp Met Ala Gln Val Thr Val Gly Pro Gly Leu Leu
705                 710                 715                 720

Gly Val Ser Thr Leu Gly Pro Lys Arg Asn Ser Met Val Leu Asp Val
                725                 730                 735
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Ala Phe Val Leu Glu Gly Ser Asp Lys Ile Gly Glu Ala Asp Phe Asn
            740                 745                 750

Arg Ser Lys Glu Phe Met Glu Glu Val Ile Gln Arg Met Asp Val Gly
        755                 760                 765

Gln Asp Ser Ile His Val Thr Val Leu Gln Tyr Ser Tyr Met Val Thr
    770                 775                 780

Val Glu Tyr Pro Phe Ser Glu Ala Gln Ser Lys Gly Asp Ile Leu Gln
785                 790                 795                 800

Arg Val Arg Glu Ile Arg Tyr Gln Gly Gly Asn Arg Thr Asn Thr Gly
                805                 810                 815

Leu Ala Leu Arg Tyr Leu Ser Asp His Ser Phe Leu Val Ser Gln Gly
            820                 825                 830

Asp Arg Glu Gln Ala Pro Asn Leu Val Tyr Met Val Thr Gly Asn Pro
        835                 840                 845

Ala Ser Asp Glu Ile Lys Arg Leu Pro Gly Asp Ile Gln Val Val Pro
    850                 855                 860

Ile Gly Val Gly Pro Asn Ala Asn Val Gln Glu Leu Glu Arg Ile Gly
865                 870                 875                 880

Trp Pro Asn Ala Pro Ile Leu Ile Gln Asp Phe Glu Thr Leu Pro Arg
                885                 890                 895

Glu Ala Pro Asp Leu Val Leu Gln Arg Cys Cys Ser Gly Glu Gly Leu
            900                 905                 910

Gln Ile Pro Thr Leu Ser Pro Ala Pro Asp Cys Ser Gln Pro Leu Asp
        915                 920                 925

Val Ile Leu Leu Leu Asp Gly Ser Ser Ser Phe Pro Ala Ser Tyr Phe
    930                 935                 940

Asp Glu Met Lys Ser Phe Ala Lys Ala Phe Ile Ser Lys Ala Asn Ile
945                 950                 955                 960

Gly Pro Arg Leu Thr Gln Val Ser Val Leu Gln Tyr Gly Ser Ile Thr
                965                 970                 975

Thr Ile Asp Val Pro Trp Asn Val Val Pro Glu Lys Ala His Leu Leu
            980                 985                 990

Ser Leu Val Asp Val Met Gln Arg  Glu Gly Gly Pro Ser  Gln Ile Gly
        995                 1000                1005

Asp Ala  Leu Gly Phe Ala Val  Arg Tyr Leu Thr Ser  Glu Met His
    1010                1015                1020

Gly Ala  Arg Pro Gly Ala Ser  Lys Ala Val Val Ile  Leu Val Thr
    1025                1030                1035

Asp Val  Ser Val Asp Ser Val  Asp Ala Ala Ala Asp  Ala Ala Arg
    1040                1045                1050

Ser Asn  Arg Val Thr Val Phe  Pro Ile Gly Ile Gly  Asp Arg Tyr
    1055                1060                1065

Asp Ala  Ala Gln Leu Arg Ile  Leu Ala Gly Pro Ala  Gly Asp Ser
    1070                1075                1080

Asn Val  Val Lys Leu Gln Arg  Ile Glu Asp Leu Pro  Thr Met Val
    1085                1090                1095

Thr Leu  Gly Asn Ser Phe Leu  His Lys Leu Cys Ser  Gly Phe Val
    1100                1105                1110

Arg Ile  Cys Met Asp Glu Asp  Gly Asn Glu Lys Arg  Pro Gly Asp
    1115                1120                1125

Val Trp  Thr Leu Pro Asp Gln  Cys His Thr Val Thr  Cys Gln Pro
    1130                1135                1140
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Asp Gly  Gln Thr Leu Leu Lys  Ser His Arg Val Asn  Cys Asp Arg
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Glu Glu  Thr Cys Gly Cys Arg  Trp Thr Cys Pro Cys  Val Cys Thr
    1175                 1180                 1185

Gly Ser  Ser Thr Arg His Ile  Val Thr Phe Asp Gly  Gln Asn Phe
    1190                 1195                 1200

Lys Leu  Thr Gly Ser Cys Ser  Tyr Val Leu Phe Gln  Asn Lys Glu
    1205                 1210                 1215

Gln Asp  Leu Glu Val Ile Leu  His Asn Gly Ala Cys  Ser Pro Gly
    1220                 1225                 1230

Ala Arg  Gln Gly Cys Met Lys  Ser Ile Glu Val Lys  His Ser Ala
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Leu Ser  Val Glu Leu His Ser  Asp Met Glu Val Thr  Val Asn Gly
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Arg Leu  Val Ser Val Pro Tyr  Val Gly Gly Asn Met  Glu Val Asn
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Val Tyr  Gly Ala Ile Met His  Glu Val Arg Phe Asn  His Leu Gly
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His Ile  Phe Thr Phe Thr Pro  Gln Asn Asn Glu Phe  Gln Leu Gln
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Leu Ser  Pro Lys Thr Phe Ala  Ser Lys Thr Tyr Gly  Leu Cys Gly
    1310                 1315                 1320

Ile Cys  Asp Glu Asn Gly Ala  Asn Asp Phe Met Leu  Arg Asp Gly
    1325                 1330                 1335

Thr Val  Thr Thr Asp Trp Lys  Thr Leu Val Gln Glu  Trp Thr Val
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Gln Arg  Pro Gly Gln Thr Cys  Gln Pro Ile Leu Glu  Glu Gln Cys
    1355                 1360                 1365

Leu Val  Pro Asp Ser Ser His  Cys Gln Val Leu Leu  Leu Pro Leu
    1370                 1375                 1380

Phe Ala  Glu Cys His Lys Val  Leu Ala Pro Ala Thr  Phe Tyr Ala
    1385                 1390                 1395

Ile Cys  Gln Gln Asp Ser Cys  His Gln Glu Gln Val  Cys Glu Val
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Ile Ala  Ser Tyr Ala His Leu  Cys Arg Thr Asn Gly  Val Cys Val
    1415                 1420                 1425

Asp Trp  Arg Thr Pro Asp Phe  Cys Ala Met Ser Cys  Pro Pro Ser
    1430                 1435                 1440

Leu Val  Tyr Asn His Cys Glu  His Gly Cys Pro Arg  His Cys Asp
    1445                 1450                 1455

Gly Asn  Val Ser Ser Cys Gly  Asp His Pro Ser Glu  Gly Cys Phe
    1460                 1465                 1470

Cys Pro  Pro Asp Lys Val Met  Leu Glu Gly Ser Cys  Val Pro Glu
    1475                 1480                 1485

Glu Ala  Cys Thr Gln Cys Ile  Gly Glu Asp Gly Val  Gln His Gln
    1490                 1495                 1500

Phe Leu  Glu Ala Trp Val Pro  Asp His Gln Pro Cys  Gln Ile Cys
    1505                 1510                 1515

Thr Cys  Leu Ser Gly Arg Lys  Val Asn Cys Thr Thr  Gln Pro Cys
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Pro Thr  Ala Lys Ala Pro Thr  Cys Gly Leu Cys Glu  Val Ala Arg
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    1535                1540                1545

Leu Arg  Gln Asn Ala Asp Gln  Cys Cys Pro Glu Tyr  Glu Cys Val
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Cys Asp  Pro Val Ser Cys Asp  Leu Pro Pro Val Pro  His Cys Glu
    1565                1570                1575

Arg Gly  Leu Gln Pro Thr Leu  Thr Asn Pro Gly Glu  Cys Arg Pro
    1580                1585                1590

Asn Phe  Thr Cys Ala Cys Arg  Lys Glu Glu Cys Lys  Arg Val Ser
    1595                1600                1605

Pro Pro  Ser Cys Pro Pro His  Arg Leu Pro Thr Leu  Arg Lys Thr
    1610                1615                1620

Gln Cys  Cys Asp Glu Tyr Glu  Cys Ala Cys Asn Cys  Val Asn Ser
    1625                1630                1635

Thr Val  Ser Cys Pro Leu Gly  Tyr Leu Ala Ser Thr  Ala Thr Asn
    1640                1645                1650

Asp Cys  Gly Cys Thr Thr Thr  Thr Cys Leu Pro Asp  Lys Val Cys
    1655                1660                1665

Val His  Arg Ser Thr Ile Tyr  Pro Val Gly Gln Phe  Trp Glu Glu
    1670                1675                1680

Gly Cys  Asp Val Cys Thr Cys  Thr Asp Met Glu Asp  Ala Val Met
    1685                1690                1695

Gly Leu  Arg Val Ala Gln Cys  Ser Gln Lys Pro Cys  Glu Asp Ser
    1700                1705                1710

Cys Arg  Ser Gly Phe Thr Tyr  Val Leu His Glu Gly  Glu Cys Cys
    1715                1720                1725

Gly Arg  Cys Leu Pro Ser Ala  Cys Glu Val Val Thr  Gly Ser Pro
    1730                1735                1740

Arg Gly  Asp Ser Gln Ser Ser  Trp Lys Ser Val Gly  Ser Gln Trp
    1745                1750                1755

Ala Ser  Pro Glu Asn Pro Cys  Leu Ile Asn Glu Cys  Val Arg Val
    1760                1765                1770

Lys Glu  Glu Val Phe Ile Gln  Gln Arg Asn Val Ser  Cys Pro Gln
    1775                1780                1785

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    1790                1795                1800

Thr Ser  Ala Cys Cys Pro Ser  Cys Arg Cys Glu Arg  Met Glu Ala
    1805                1810                1815

Cys Met  Leu Asn Gly Thr Val  Ile Gly Pro Gly Lys  Thr Val Met
    1820                1825                1830

Ile Asp  Val Cys Thr Thr Cys  Arg Cys Met Val Gln  Val Gly Val
    1835                1840                1845

Ile Ser  Gly Phe Lys Leu Glu  Cys Arg Lys Thr Thr  Cys Asn Pro
    1850                1855                1860

Cys Pro  Leu Gly Tyr Lys Glu  Glu Asn Asn Thr Gly  Glu Cys Cys
    1865                1870                1875

Gly Arg  Cys Leu Pro Thr Ala  Cys Thr Ile Gln Leu  Arg Gly Gly
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Gln Ile  Met Thr Leu Lys Arg  Asp Glu Thr Leu Gln  Asp Gly Cys
    1895                1900                1905

Asp Thr  His Phe Cys Lys Val  Asn Glu Arg Gly Glu  Tyr Phe Trp
    1910                1915                1920

Glu Lys  Arg Val Thr Gly Cys  Pro Pro Phe Asp Glu  His Lys Cys
    1925                1930                1935
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Asp Thr  Cys Glu Glu Pro Glu  Cys Asn Asp Ile Thr  Ala Arg Leu
    1955                1960                1965

Gln Tyr  Val Lys Val Gly Ser  Cys Lys Ser Glu Val  Glu Val Asp
    1970                1975                1980

Ile His  Tyr Cys Gln Gly Lys  Cys Ala Ser Lys Ala  Met Tyr Ser
    1985                1990                1995

Ile Asp  Ile Asn Asp Val Gln  Asp Gln Cys Ser Cys  Cys Ser Pro
    2000                2005                2010

Thr Arg  Thr Glu Pro Met Gln  Val Ala Leu His Cys  Thr Asn Gly
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caactccatc actaggggtt cctggagggg tggagtcgtg acccctaaaa tgggcaaaca    180

ttgcaagcag caaacagcaa acacacagcc ctccctgcct gctgaccttg gagctggggc    240

agaggtcaga gacctctctg ggcccatgcc acctccaaca tccactcgac cccttggaat    300

ttcggtggag aggagcagag gttgtcctgg cgtggtttag gtagtgtgag aggggaatga    360

ctcctttcgg taagtgcagt ggaagctgta cactgcccag gcaaagcgtc cgggcagcgt    420

aggcgggcga ctcagatccc agccagtgga cttagcccct gtttgctcct ccgataactg    480

gggtgacctt ggttaatatt caccagcagc ctcccccgtt gcccctctgg atccactgct    540

taaatacgga cgaggacagg gccctgtctc ctcagcttca ggcaccacca ctgacctggg    600

acagtgaatc cggactctaa ggtaaatata aaatttttaa gtgtataatg tgttaaacta    660

ctgattctaa ttgtttctct cttttagatt ccaacctttg gaactgaatt ctagaccacc    720

atg cag agg gtg aac atg atc atg gct gag agc cct ggc ctg atc acc      768
Met Gln Arg Val Asn Met Ile Met Ala Glu Ser Pro Gly Leu Ile Thr
1               5                  10                  15

atc tgc ctg ctg ggc tac ctg ctg tct gct gag tgc act gtg ttc ctg      816
Ile Cys Leu Leu Gly Tyr Leu Leu Ser Ala Glu Cys Thr Val Phe Leu
            20                  25                  30

gac cat gag aat gcc aac aag atc ctg aac agg ccc aag aga tac aac      864
Asp His Glu Asn Ala Asn Lys Ile Leu Asn Arg Pro Lys Arg Tyr Asn
        35                  40                  45

tct ggc aag ctg gag gag ttt gtg cag ggc aac ctg gag agg gag tgc      912
Ser Gly Lys Leu Glu Glu Phe Val Gln Gly Asn Leu Glu Arg Glu Cys
    50                  55                  60
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atg gag gag aag tgc agc ttt gag gag gcc agg gag gtg ttt gag aac      960
Met Glu Glu Lys Cys Ser Phe Glu Glu Ala Arg Glu Val Phe Glu Asn
65                  70                  75                  80

act gag agg acc act gag ttc tgg aag cag tat gtg gat ggg gac cag     1008
Thr Glu Arg Thr Thr Glu Phe Trp Lys Gln Tyr Val Asp Gly Asp Gln
                85                  90                  95

tgt gag agc aac ccc tgc ctg aat ggg ggc agc tgc aag gat gac atc     1056
Cys Glu Ser Asn Pro Cys Leu Asn Gly Gly Ser Cys Lys Asp Asp Ile
            100                 105                 110

aac agc tat gag tgc tgg tgc ccc ttt ggc ttt gag ggc aag aac tgt     1104
Asn Ser Tyr Glu Cys Trp Cys Pro Phe Gly Phe Glu Gly Lys Asn Cys
        115                 120                 125

gag ctg gat gtg acc tgc aac atc aag aat ggc aga tgt gag cag ttc     1152
Glu Leu Asp Val Thr Cys Asn Ile Lys Asn Gly Arg Cys Glu Gln Phe
    130                 135                 140

tgc aag aac tct gct gac aac aag gtg gtg tgc agc tgc act gag ggc     1200
Cys Lys Asn Ser Ala Asp Asn Lys Val Val Cys Ser Cys Thr Glu Gly
145                 150                 155                 160

tac agg ctg gct gag aac cag aag agc tgt gag cct gct gtg cca ttc     1248
Tyr Arg Leu Ala Glu Asn Gln Lys Ser Cys Glu Pro Ala Val Pro Phe
                165                 170                 175

cca tgt ggc aga gtg tct gtg agc cag acc agc aag ctg acc agg gct     1296
Pro Cys Gly Arg Val Ser Val Ser Gln Thr Ser Lys Leu Thr Arg Ala
            180                 185                 190

gag gct gtg ttc cct gat gtg gac tat gtg aac agc act gag gct gaa     1344
Glu Ala Val Phe Pro Asp Val Asp Tyr Val Asn Ser Thr Glu Ala Glu
        195                 200                 205

acc atc ctg gac aac atc acc cag agc acc cag agc ttc aat gac ttc     1392
Thr Ile Leu Asp Asn Ile Thr Gln Ser Thr Gln Ser Phe Asn Asp Phe
    210                 215                 220

acc agg gtg gtg ggg ggg gag gat gcc aag cct ggc cag ttc ccc tgg     1440
Thr Arg Val Val Gly Gly Glu Asp Ala Lys Pro Gly Gln Phe Pro Trp
225                 230                 235                 240

caa gtg gtg ctg aat ggc aag gtg gat gcc ttc tgt ggg ggc agc att     1488
Gln Val Val Leu Asn Gly Lys Val Asp Ala Phe Cys Gly Gly Ser Ile
                245                 250                 255

gtg aat gag aag tgg att gtg act gct gcc cac tgt gtg gag act ggg     1536
Val Asn Glu Lys Trp Ile Val Thr Ala Ala His Cys Val Glu Thr Gly
            260                 265                 270

gtg aag atc act gtg gtg gct ggg gag cac aac att gag gag act gag     1584
Val Lys Ile Thr Val Val Ala Gly Glu His Asn Ile Glu Glu Thr Glu
        275                 280                 285

cac act gag cag aag agg aat gtg atc agg atc atc ccc cac cac aac     1632
His Thr Glu Gln Lys Arg Asn Val Ile Arg Ile Ile Pro His His Asn
    290                 295                 300

tac aat gct gcc atc aac aag tac aac cat gac att gcc ctg ctg gag     1680
Tyr Asn Ala Ala Ile Asn Lys Tyr Asn His Asp Ile Ala Leu Leu Glu
305                 310                 315                 320

ctg gat gag ccc ctg gtg ctg aac agc tat gtg acc ccc atc tgc att     1728
Leu Asp Glu Pro Leu Val Leu Asn Ser Tyr Val Thr Pro Ile Cys Ile
                325                 330                 335

gct gac aag gag tac acc aac atc ttc ctg aag ttt ggc tct ggc tat     1776
Ala Asp Lys Glu Tyr Thr Asn Ile Phe Leu Lys Phe Gly Ser Gly Tyr
            340                 345                 350

gtg tct ggc tgg ggc agg gtg ttc cac aag ggc agg tct gcc ctg gtg     1824
Val Ser Gly Trp Gly Arg Val Phe His Lys Gly Arg Ser Ala Leu Val
        355                 360                 365

ctg cag tac ctg agg gtg ccc ctg gtg gac agg gcc acc tgc ctg agg     1872
Leu Gln Tyr Leu Arg Val Pro Leu Val Asp Arg Ala Thr Cys Leu Arg
    370                 375                 380
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agc acc aag ttc acc atc tac aac aac atg ttc tgt gct ggc ttc cat     1920
Ser Thr Lys Phe Thr Ile Tyr Asn Asn Met Phe Cys Ala Gly Phe His
385                 390                 395                 400

gag ggg ggc agg gac agc tgc cag ggg gac tct ggg ggc ccc cat gtg     1968
Glu Gly Gly Arg Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro His Val
                405                 410                 415

act gag gtg gag ggc acc agc ttc ctg act ggc atc atc agc tgg ggg     2016
Thr Glu Val Glu Gly Thr Ser Phe Leu Thr Gly Ile Ile Ser Trp Gly
            420                 425                 430

gag gag tgt gcc atg aag ggc aag tat ggc atc tac acc aaa gtc tcc     2064
Glu Glu Cys Ala Met Lys Gly Lys Tyr Gly Ile Tyr Thr Lys Val Ser
        435                 440                 445

aga tat gtg aac tgg atc aag gag aag acc aag ctg acc tgactcgatg      2113
Arg Tyr Val Asn Trp Ile Lys Glu Lys Thr Lys Leu Thr
    450                 455                 460

ctttatttgt gaaatttgtg atgctattgc tttatttgta accattataa gctgcaataa   2173

acaagttaac aacaacaatt gcattcattt tatgtttcag gttcaggggg aggtgtggga   2233

ggtttttttaa actagtccac tccctctctg cgcgctcgct cgctcactga ggccgggcga   2293

ccaaaggtcg cccgacgccc gggctttgcc cgggcggcct cagtgagcga gcgagcgcgc   2353

agagaggga                                                           2362
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<210> SEQ ID NO 3
<211> LENGTH: 461
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 3

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1               5                   10                  15

Ile Cys Leu Leu Gly Tyr Leu Leu Ser Ala Glu Cys Thr Val Phe Leu
            20                  25                  30

Asp His Glu Asn Ala Asn Lys Ile Leu Asn Arg Pro Lys Arg Tyr Asn
        35                  40                  45

Ser Gly Lys Leu Glu Glu Phe Val Gln Gly Asn Leu Glu Arg Glu Cys
    50                  55                  60

Met Glu Glu Lys Cys Ser Phe Glu Glu Ala Arg Glu Val Phe Glu Asn
65                  70                  75                  80

Thr Glu Arg Thr Thr Glu Phe Trp Lys Gln Tyr Val Asp Gly Asp Gln
                85                  90                  95

Cys Glu Ser Asn Pro Cys Leu Asn Gly Gly Ser Cys Lys Asp Asp Ile
            100                 105                 110

Asn Ser Tyr Glu Cys Trp Cys Pro Phe Gly Phe Glu Gly Lys Asn Cys
        115                 120                 125

Glu Leu Asp Val Thr Cys Asn Ile Lys Asn Gly Arg Cys Glu Gln Phe
    130                 135                 140

Cys Lys Asn Ser Ala Asp Asn Lys Val Val Cys Ser Cys Thr Glu Gly
145                 150                 155                 160

Tyr Arg Leu Ala Glu Asn Gln Lys Ser Cys Glu Pro Ala Val Pro Phe
                165                 170                 175

Pro Cys Gly Arg Val Ser Val Ser Gln Thr Ser Lys Leu Thr Arg Ala
            180                 185                 190

Glu Ala Val Phe Pro Asp Val Asp Tyr Val Asn Ser Thr Glu Ala Glu
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        195                 200                 205

Thr Ile Leu Asp Asn Ile Thr Gln Ser Thr Gln Ser Phe Asn Asp Phe
    210                 215                 220

Thr Arg Val Val Gly Gly Glu Asp Ala Lys Pro Gly Gln Phe Pro Trp
225                 230                 235                 240

Gln Val Val Leu Asn Gly Lys Val Asp Ala Phe Cys Gly Gly Ser Ile
                245                 250                 255

Val Asn Glu Lys Trp Ile Val Thr Ala Ala His Cys Val Glu Thr Gly
            260                 265                 270

Val Lys Ile Thr Val Val Ala Gly Glu His Asn Ile Glu Glu Thr Glu
        275                 280                 285

His Thr Glu Gln Lys Arg Asn Val Ile Arg Ile Ile Pro His His Asn
    290                 295                 300

Tyr Asn Ala Ala Ile Asn Lys Tyr Asn His Asp Ile Ala Leu Leu Glu
305                 310                 315                 320

Leu Asp Glu Pro Leu Val Leu Asn Ser Tyr Val Thr Pro Ile Cys Ile
                325                 330                 335

Ala Asp Lys Glu Tyr Thr Asn Ile Phe Leu Lys Phe Gly Ser Gly Tyr
            340                 345                 350

Val Ser Gly Trp Gly Arg Val Phe His Lys Gly Arg Ser Ala Leu Val
        355                 360                 365

Leu Gln Tyr Leu Arg Val Pro Leu Val Asp Arg Ala Thr Cys Leu Arg
    370                 375                 380

Ser Thr Lys Phe Thr Ile Tyr Asn Asn Met Phe Cys Ala Gly Phe His
385                 390                 395                 400

Glu Gly Gly Arg Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro His Val
                405                 410                 415

Thr Glu Val Glu Gly Thr Ser Phe Leu Thr Gly Ile Ile Ser Trp Gly
            420                 425                 430

Glu Glu Cys Ala Met Lys Gly Lys Tyr Gly Ile Tyr Thr Lys Val Ser
        435                 440                 445

Arg Tyr Val Asn Trp Ile Lys Glu Lys Thr Lys Leu Thr
    450                 455                 460


<210> SEQ ID NO 4
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 4

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<210> SEQ ID NO 5
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      primer

<400> SEQUENCE: 5

tcatggaagc cagcacagaa catg                                          24
```

What is claimed is:

1. A pharmaceutical product comprising:

a pharmaceutical composition comprising an immune globulin infusion; Antihemophilic Factor (Recombinant); recombinant von Willebrand factor (rVWF); or sscAAV2/8-LP1-hFIXco, a self-complementary adeno-associated viral vector containing a liver-specific human factor IX expression cassette, and a pharmaceutically acceptable excipient contained within a glass pharmaceutical container comprising a glass composition comprising:

SiO_2 in an amount greater than or equal to about 72 mol. % and less than or equal to about 78 mol. %;

alkaline earth oxide comprising both MgO and CaO, wherein CaO is present in an amount up to about 1.0 mol. %, and a ratio (CaO (mol. %)/(CaO (mol. %)+MgO (mol. %))) is less than or equal to 0.5;

X mol. % Al_2O_3, wherein X is greater than or equal to about 5 mol. % and less than or equal to about 7 mol. %;

Y mol. % alkali oxide, wherein the alkali oxide comprises Na_2O in an amount greater than about 8 mol. %; and

a ratio of a concentration of B_2O_3 (mol. %) in the glass container to (Y mol. %−X mol. %) is less than or equal to 0.3.

2. The pharmaceutical product of claim **1**, wherein the pharmaceutical container comprises a compressive stress greater than or equal to 150 MPa.

3. The pharmaceutical product of claim **1**, wherein the pharmaceutical container comprises a compressive stress greater than or equal to 250 MPa.

4. The pharmaceutical product of claim **1**, wherein the pharmaceutical container comprises a depth of layer greater than 30 μm.

5. The pharmaceutical product of claim **1**, wherein the pharmaceutical product has increased stability, product integrity, or efficacy as compared to the pharmaceutical composition not contained within the glass pharmaceutical container.

6. The pharmaceutical product of claim **1** wherein the glass pharmaceutical container has a compressive stress greater than or equal to 150 MPa and a depth of layer greater than 10 μm, and wherein the pharmaceutical product comprises increased stability, product integrity, or efficacy as compared to the pharmaceutical composition not contained within the glass pharmaceutical container.

7. The pharmaceutical product of claim **1** wherein the glass pharmaceutical container is substantially free of boron, and wherein the pharmaceutical product comprises increased stability, product integrity, or efficacy as compared to the pharmaceutical composition not contained within the glass pharmaceutical container.

8. The pharmaceutical product of claim **7**, wherein the glass pharmaceutical container comprises a compressive stress greater than or equal to 150 MPa and a depth of layer greater than 25 μm.

9. The pharmaceutical product of claim **8**, wherein the glass pharmaceutical container comprises a compressive stress greater than or equal to 300 MPa and a depth of layer greater than 35 μm.

10. The pharmaceutical product of claim **7**, wherein said glass pharmaceutical container comprises a substantially homogeneous inner layer.

11. The pharmaceutical product of claim **10**, wherein said glass pharmaceutical container comprises a compressive stress greater than or equal to 150 MPa and a depth of layer greater than 25 μm.

12. The pharmaceutical product of claim **1**, wherein the pharmaceutical container comprises an internal homogeneous layer.

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